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TRANSFORMACIONES DE LA MATERIA ORGÁNICA EN SUELOS ENMENDADOS CON LODOS DE DEPURADORAS URBANAS: IMPLICACIONES AGRONÓMICAS Y AMBIENTALES

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TRANSFORMACIONES DE LA MATERIA ORGÁNICA EN SUELOS ENMENDADOS CON LODOS DE DEPURADORAS URBANAS: IMPLICACIONES AGRONÓMICAS Y AMBIENTALES

Memoria que presenta D. José Manuel Fernández Arroyo para optar al grado de Doctor en Ciencias

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A mis padres

Son cosas chiquitas, no acaban con la pobreza, no sacan del subdesarrollo, no socializan los medios, no expropian las cuevas de Ali Baba. Pero quizás desencadenen la alegría del hacer, y la traducen en actos. Al fin y al cabo, actuar sobre la realidad y cambiarla, aunque solo sea un poquito, es la única manera de probar que la Realidad es Transformable.

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ÍNDICE

RESUMEN

ABSTRACT

RESUMEN

Este trabajo aborda el estudio del uso seguro y eficiente en agricultura de lodos de depuradora compostados y secados térmicamente, con objeto de proporcionar una solución adecuada a la creciente producción de este residuo y mejorar la fertilidad de los suelos de una manera sostenible. Se llevó a cabo un experimento de laboratorio y otro de campo con el fin de estudiar los tipos y la extensión de los efectos a largo plazo inducidos por la aplicación de estas enmiendas sobre las propiedades químicas, biológicas y bioquímicas del suelo así como su influencia en las transformaciones de la materia orgánica, prestando especial atención a la fracción de los ácidos húmicos.

En el experimento de laboratorio, un suelo sin enmendar o enmendado con lodo compostado o secado térmicamente fue incubado en condiciones controladas de humedad y temperatura durante 60 días. Las emisiones de CO_2 de las muestras fueron medidas periódicamente y los resultados se ajustaron a seis modelos cinéticos distintos. El diseño del experimento de campo incluye suelos sin enmendar (control) o enmendados con fertilizante mineral o con lodos compostados o secados térmicamente en dosis de 20 y 80 t ha^{-1}. Después del primer año de aplicación de los lodos, cada parcela se dividió por la mitad en dos subparcelas. En una de ellas no se volvió a añadir mas residuo en los siguientes 3 años, con el fin de evaluar los efectos residuales de la primera aplicación. En el segundo grupo de subparcelas, se evaluaron los efectos acumulativos de las enmiendas aplicándolas de nuevo cada año. Además todos los suelos fueron cultivados con cebada (*Hordeum vulgare* L.).

El estudio de las cinéticas de mineralización del C en estos suelos revela una mayor presencia de compuestos fácilmente biodegradables en los lodos secados térmicamente que en los compostados, aunque estos últimos, siguen un patrón de mineralización muy similar al observado en el suelo control. La adición de ambos lodos induce varias modificaciones en las propiedades del suelo, incluido un incremento del pH, de la conductividad eléctrica, del C orgánico total, del N total y del P asimilable. En los tratamientos acumulativos, las dosis altas de lodos causan un incremento de los contenidos de C orgánico total y de la biomasa

microbiana así como un aumento de las actividades enzimáticas. Sin embargo, los mejores rendimientos de cultivo se obtienen en los suelos enmendados una única vez, particularmente en aquellos a los que se les aplicó lodo compostado.

En general, los resultados obtenidos indican que ambas clases de lodos mejoran los niveles de materia orgánica del suelo y de sus fracciones humificadas, aunque el lodo compostado puede considerarse más eficiente como enmienda orgánica que el secado térmicamente, que puede presentar problemas de estabilidad. Los análisis muestran importantes diferencias en la composición química y estructural de los ácidos húmicos de ambos lodos, con una importante presencia de compuestos alifáticos y de cadenas largas con grupos nitrogenados. Después de la adición anual de las dosis altas de 80 t ha^{-1}, las sustancias húmicas del suelo muestran un descenso en el contenido de grupos funcionales ácidos y un ligero incremento de las afinidades protónicas de los grupos carboxílicos y fenólicos. Además, los ácidos húmicos del suelo, que originariamente presentaban unas características marcadamente aromáticas, muestran mayor cantidad de constituyentes alifáticos y proteicos, especialmente en los suelos enmendados con lodos secados térmicamente, lo que sugiere que los ácido húmicos de los lodos son parcialmente incorporados a los del suelo.

ABSTRACT

This research deals with the study of the safe and efficient use of composted and thermally-dried sewage sludges in agriculture with the aim of providing a suitable solution for the increasing production of this waste and improving soil fertility in a sustainable way. Both a laboratory and a field experiment were carried out in order to study the type and extent of long-term effects induced by the application of these amendments on chemical, biological and biochemical properties of the soil as well as on soil organic matter transformations, focusing special attention on the humic acid fraction.

In the laboratory experiment, an arid soil either unamended or amended with composted sewage sludge or thermally-dried sewage sludge was incubated under controlled conditions of moisture and temperature for 60 days. The CO_2 emission from the samples was periodically measured and the data were fitted to six different kinetic models. The field experimental design included soil either unamended (control) or amended with mineral fertilizer, composted and thermally-dried sewage sludges at rates of 20 and 80 t ha^{-1}. After the first year of sewage sludge application, each plot was divided into two subplots. In one subplot group, no additional application of wastes was made in the following 3 years, and the residual effect of the first applications was evaluated. In the second subplot group, the cumulative effect of sludge amendments was evaluated by applying these amendments also in the following three consecutive years. All the plots were crop with barley (*Hordeum vulgare* L.).

The study of C mineralization kinetics in these soils reveals a larger presence of easily biodegradable compounds in thermally dried sewage sludges than in the composted ones, which in turn presents a C mineralization pattern very similar to that of the unamended soil. Amendment with both sludges induces a number of modifications in soil properties, including an increase of pH, electrical conductivity, total organic C, total N, and available P. In the cumulative experiment, high doses of sewage sludge cause an increase of the total organic and biomass C contents as well as of enzyme activities. However, the best results for

barley yield are obtained for the soils amended once with sewage sludge, particularly those amended with the composted sewage sludge.

In general, results obtained in the cumulative and residual experiments indicate that both kind of sludges improve soil organic matter levels and its humified fractions, although the composted sewage sludge can be considered as a more efficient organic amendment than the thermally-dried sewage sludge, which may present problems of stability. Results show important differences in the chemical composition and structure of the humic acids from the two sewage sludges, with an important presence of alkyl compounds and long chain N-containing structures in the thermally dried sewage sludge humic acids. After their annual addition at a rate of 80 t ha^{-1}, soil humic substances feature a decrease of acidic functional group contents and a slight increase of proton binding affinities of carboxylic- and phenolic-type groups. Specifically, soil humic acids, that originally presented a marked aromatic character, exhibited an enrichment in aliphatic and proteinaceous constituents, especially those amended with thermally-dried sewage sludge, which suggests that sludge humic acids are partially incorporated into soil humic acids.

I. INTRODUCCIÓN

ÍNDICE

I.1. EL SUELO

I.1.1. Valor ambiental

El suelo es un sistema natural, organizado e independiente, cuya formación se debe a la acción conjunta del clima, los organismos, la vegetación, el relieve y el tiempo sobre la roca madre, constituye una matriz de componentes orgánicos y minerales que engloba una red porosa por donde circulan líquidos y gases, albergando numerosas poblaciones de organismos vivos en una situación de equilibrio dinámico (Agencia Europea de Medio Ambiente, 2004).

En 1995, la Sociedad Americana de la Ciencia del Suelo (SSSA) definió la calidad del suelo como "la capacidad de un tipo específico de suelo para desempeñar sus funciones dentro de los límites de un ecosistema natural o manejado, sosteniendo la producción de las plantas y de los animales, manteniendo o realzando la calidad del agua y del aire y apoyando la salud y la ocupación humana". Comenzaba así a generalizarse una nueva concepción del suelo en donde destaca la importancia de sus funciones para el desarrollo social y económico superando una concepción más relacionada con sus propiedades y con los aspectos que condicionan la edafogénesis.

Las funciones del suelo son muy diversas y pueden referirse a aspectos ecológicos, técnicos, industriales, socioeconómicos y culturales. Entre las principales destacan las siguientes (Seybold et al., 1998; Porta, 2005):

- **Función productiva**: ser suministro de nutrientes, aire y agua a las plantas y sostener la actividad, diversidad y productividad biológica de los ecosistemas.
- **Función Hidrológica**: regular la infiltración, almacenamiento y flujo de agua superficiales y subsuperficiales junto a su calidad.
- **Función Medioambiental**: filtrar, amortiguar, degradar, inmovilizar y reducir la toxicidad de compuestos orgánicos e inorgánicos, incluyendo subproductos urbanos e industriales y deposiciones atmosféricas.

- **Función Biológica**: hábitat de diferentes organismos, conservación de biodiversidad y reserva de genes.
- **Función Biogeoquímica**: almacenar y posibilitar el ciclo biogeoquímico de nutrientes y otros elementos con efectos sobre el equilibrio energético global.
- **Función Socioeconómica**: servir de base espacial para las actividades agrícolas, asentamientos humanos e infraestructuras así como ser fuente de materias primas.

A pesar de que el suelo, en principio, es un recurso natural renovable, su génesis es un proceso muy lento, por lo que debe ser tratado como "no renovable"; sin embargo, debido a su extraordinaria capacidad para biodegradar residuos, el hecho de que el tiempo de permanencia de sustancias contaminantes sea mucho mayor en él que en otros medios como el aire o el agua y el retraso que existe normalmente en la detección de efectos nocivos, explica que la preocupación por su buen estado no haya sido excesiva. Así, en Europa sólo en fechas recientes se ha tomado conciencia de la necesidad de preservar el suelo (Agencia Europea de Medio Ambiente, 1998).

El suelo es necesario para el desarrollo de los cultivos, siendo un componente esencial de todos los ecosistemas terrestres; por consiguiente, es de vital importancia para la humanidad y para el mantenimiento de un medioambiente saludable. Por ello, la conservación del suelo para el desarrollo de sus funciones es un asunto que implica la necesidad de establecer estrategias que eviten, o al menos reduzcan, la degradación de los sistemas edáficos a escala global y local, contribuyendo con ello a un desarrollo sostenible. En este contexto, hay que considerar el papel fundamental del establecimiento de prácticas agrícolas adecuadas, que en el marco de una gestión integral de los recursos naturales, permitan alcanzar la máxima productividad sin comprometer la fertilidad del suelo para las generaciones futuras.

I.1.2. Degradación del suelo

La degradación es un problema multidisciplinar y multicasual que generalmente esconde un conjunto de procesos interrelacionados (físicos, químicos, etc.) que se

manifiestan a diferentes niveles de resolución, tanto espacial como temporal (López-Bermúdez, 2006). La degradación del suelo puede definirse como "la alteración del equilibrio existente entre sus constituyentes debido a los cambios experimentados en sus propiedades físicas, químicas, biológicas o bioquímicas, que conduce a la pérdida o disminución de su fertilidad y que disminuye la capacidad actual o futura del suelo para generar, en términos de calidad y cantidad, bienes o servicios" (González-Quiñones, 2006).

Los principales tipos de degradación a nivel mundial son la erosión hídrica (56%), la erosión eólica (28%), la degradación química (12%) y la degradación física (4%) (GACGC, 1994); en concreto, la inducida por el hombre ha afectado a casi 2000 millones de hectáreas a escala mundial, representando aproximadamente un 15% del área total de los suelos (GLASOD, 1990).

Aunque en Europa los problemas son, en general, menos graves que en otras partes del mundo, existen extensas zonas afectadas por la erosión eólica e hídrica, la anegación y salinización, la compactación, la acidificación, la pérdida de materia orgánica y microorganismos, el agotamiento de nutrientes y la acumulación de contaminantes (Agencia Europea de Medio Ambiente, 2004).

En concreto, en la región mediterránea, los suelos manifiestan procesos de degradación debidos a factores climáticos de marcada aridez, con temperaturas elevadas, escasez e irregularidad de las precipitaciones, fuerte insolación y una evapotranspiración muy intensa, que favorecen el deterioro de sus propiedades, observándose procesos irreversibles de desertificación en el medio (Zalidis et al., 2002). Además, estas condiciones ambientales favorecen y aceleran los procesos de mineralización de la materia orgánica existentes en el sistema, lo que les confiere una escasa capacidad de retención hídrica y provoca una disminución progresiva de la cobertura vegetal, favoreciendo los procesos de erosión (Dunjó et al., 2003).

Entre las acciones antrópicas responsables de la degradación de los suelos cabe destacar aquellas relacionadas con la agricultura que en las últimas décadas se ha dirigido hacia un aumento de la productividad, intensificando al máximo los recursos existentes, y

empleando para ello distintas prácticas agrícolas que han resultado, a largo plazo, perjudiciales para el suelo (utilización de monocultivos, abuso de fertilizantes minerales y plaguicidas, maquinaria agrícola pesada, sobrepastoreo, etc.). En gran parte de los suelos del área mediterránea la agricultura intensiva llevada a cabo ha forzado el empleo de técnicas inadecuadas de manejo, dando lugar a una pérdida de su calidad y de su fertilidad que ha llevado a su posterior abandono (Dunjó et al., 2003). Sin embargo, el mantenimiento de la capacidad productiva de estos agroecosistemas a corto y a largo plazo es de vital importancia y, para ello, es necesario que los procesos de degradación del suelo, naturales o antrópicos, se equilibren mediante la aplicación de prácticas de conservación en las que la materia orgánica tiene una importancia decisiva (Hernández, 2006).

I.2. LA MATERIA ORGÁNICA

El término "materia orgánica del suelo" se refiere a todas las sustancias presentes en el mismo que contienen carbono orgánico, incluyendo restos de tejidos vegetales y animales inalterados (morfológicamente identificables), materiales en vía de descomposición y sus productos de descomposición parcial (sustancias no húmicas como carbohidratos, lípidos y aminoácidos), materiales de neoformación producidos mediante procesos de resíntesis y recombinación (sustancias húmicas) y la biomasa microbiana (bacterias, actinomicetos, hongos, protozoos y nemátodos).

La materia orgánica del suelo tiene su origen en el carbono atmosférico fijado a través de las reacciones de fotosíntesis y que es incorporado fundamentalmente con los restos vegetales y los exudados radiculares; otras fuentes, aunque de menor importancia cuantitativa, son de origen animal y microbiano (**Figura I.1**). Los procesos de mineralización devuelven el carbono a la atmósfera como dióxido de carbono principalmente, mientras que una fracción del mismo es asimilada en tejidos microbianos (biomasa del suelo) y parte se transforma a través de los procesos de humificación en formas estables (sustancias húmicas), que se pueden también mineralizar aunque de manera más lenta.

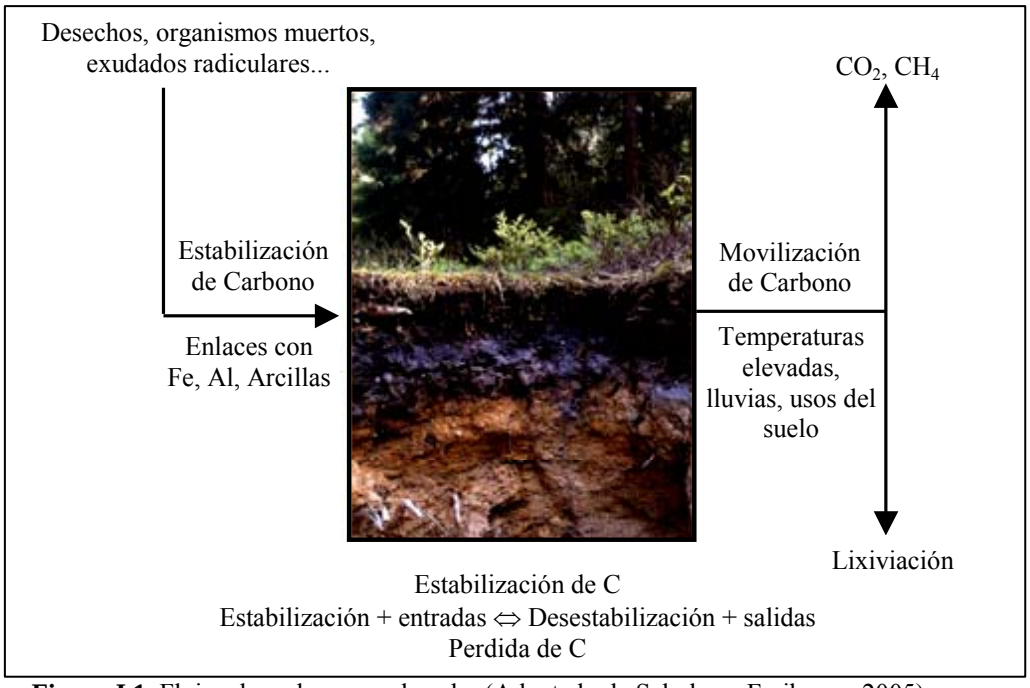

Figura I.1. Flujos de carbono en el suelo. (Adaptado de Schulze y Freibauer, 2005)

En los suelos se almacena cerca del 50% de la masa total del carbono orgánico de la superficie terrestre (Senesi y Loffredo, 1999); los contenidos de materia orgánica varían desde menos del 1% en suelos arenosos y desérticos al 1-5% en los horizontes superficiales de suelo minerales y agrícolas, y hasta casi un 100% en los orgánicos (Schnitzer, 1991). Las principales funciones de la materia orgánica del suelo se enumeran en la **Tabla I.1**.

La materia orgánica de un ecosistema maduro presenta un estado en evolución permanente, que tiende a alcanzar un equilibrio dinámico cuando las entradas de carbono al suelo por los aportes de los residuos vegetales y animales son iguales a las salidas debidas a los procesos de mineralización y lixiviación de sus fracciones solubles (Schulze y Freibauer, 2005). En un ecosistema en equilibrio dinámico, el contenido de materia orgánica del suelo se mantiene en un nivel más o menos constante mientras este ciclo se mantiene activo. Un cambio inadecuado en el manejo o en el uso del suelo puede provocar un desajuste de este estado de equilibrio y provocar grandes pérdidas de materia orgánica. Como dato relevante a este respecto, hay que indicar que la mayoría del carbono que se ha liberado a la atmósfera

en los últimos dos siglos ha tenido su origen en la conversión de suelos de praderas y bosques a suelos agrícolas (Instituto de Recursos Mundiales, 2002).

Tabla I.1: Propiedades y funciones de la materia orgánica en el suelo.

PROPIEDADES DE LA MATERIA ORGÁNICA	FUNCIÓN EN EL SUELO
Color oscuro. (Stevenson, 1994)	Favorece el calentamiento del suelo.
Capacidad de retención hídrica. (Piccolo y Mbagwu, 1994)	Aumenta la retención hídrica. Reduce la compactación del suelo.
Capacidad de formar enlaces con componentes minerales. (Stevenson, 1994)	Actúa como cementante de partículas, favoreciendo la formación de agregados estables. Estabiliza la estructura. Aumenta la permeabilidad. Permite los intercambios de gases.
Capacidad tampón de pH. (Makarov, 1994)	Estabiliza el pH del suelo, protegiendo delicados equilibrios químicos y biológicos.
Alta capacidad de intercambio catiónico. (Stevenson, 1994)	Contribuye sustancialmente a la capacidad de intercambio catiónico del suelo, favoreciendo la nutrición mineral.
Reserva de nutrientes. (Schulten y Schnitzer, 1998)	Libera NH_4^+, NO_3^-, PO_4^{3-}, SO_4^{2-}. Garantiza una fuente continua de elementos nutritivos.
Capacidad de formar complejos estables con distintos cationes polivalentes. (Senesi, 1992)	Condiciona la solubilidad y la disponibilidad de muchos micronutrientes.
Capacidad de interaccionar con fitofármacos y sustancias xenobióticas. (Senesi y Miano, 1995)	Condiciona la bioactividad, biodegradabilidad y la persistencia en el suelo.

Como se ha comentado con anterioridad, el término materia orgánica engloba en su sentido más amplio todos los materiales orgánicos contenidos en el suelo, incluyendo los organismos vivos, sus restos no descompuestos y parcialmente descompuestos y los

productos resintetizados química y microbiológicamente resistentes al ataque biológico (Senesi y Loffredo, 1999). Utilizando una definición más restrictiva, la materia orgánica del suelo o humus incluye un amplio espectro de constituyentes orgánicos, muchos de los cuales tienen su contrapartida en los tejidos biológicos (excluyendo los tejidos animales y vegetales no descompuestos), sus productos de descomposición parcial y la biomasa del suelo (Stevenson, 1994).

Para simplificar este sistema tan complejo, la materia orgánica del suelo se divide generalmente en dos grupos: sustancias no húmicas, que incluyen los compuestos orgánicos pertenecientes a las clases químicamente conocidas (aminoácidos, glúcidos, lípidos, etc.) y que no se encuentran únicamente en los suelos; y sustancias húmicas que es la fracción cuantitativamente más importante. Las sustancias húmicas se forman en el suelo por reacciones de síntesis secundarias (humificación) durante los procesos de descomposición y transformación de biomoléculas que se originan a partir de los organismos muertos y a través de la actividad de los microorganismos siendo relativamente resistentes al ataque microbiano (Senesi y Loffredo, 1999). Debido a la gran complejidad de las sustancias húmicas, causada por la variabilidad de factores que intervienen en su formación (material original, microorganismos del suelo, condiciones ambientales, etc.), éstas han sido definidas en base a las características observadas en los procesos de su fraccionamiento y no en términos químicos específicos; en este sentido, es posible realizar su fraccionamiento en distintos componentes que presentan propiedades físicas y químicas distintas (**Figura I.2**).

Entre las fracciones de la materia orgánica humificada del suelo destacan la humina (fracción insoluble a cualquier valor de pH) y los ácidos húmicos y fúlvicos, que se distinguen entre sí por su diferente solubilidad en medios acuosos. Los primeros son solubles en agua a pH ligeramente alcalino y precipitan con la acidificación del medio, mientras que los de ácidos fúlvicos son solubles en agua en todo rango de pH.

La gran complejidad que presentan las sustancias húmicas, en cuanto a su composición y estructura, ha hecho necesarios grandes esfuerzos para conocer su composición que varía dependiendo de su origen, método de extracción y otros parámetros. Los análisis elementales de estos compuestos muestran que, en general, el 98-100% de sus

elementos (libres de cenizas) son C, H, O, N, S y P . La reactividad de las sustancias húmicas y por tanto, sus efectos sobre el suelo y las plantas, está estrechamente relacionada con el tipo y contenido de grupos funcionales de las mismas. La mayor parte son de tipo oxigenado: carboxilos, alcoholes, hidroxílicos fenólicos y carbonilos aunque también se pueden encontrar grupos nitrogenados, éteres, hidroxiquinonas, lactonas, etc. Los ácidos húmicos difieren principalmente de los fúlvicos en su mayor masa molecular, contenido de carbono y grado de policondensación y en su menor concentración de grupos funcionales oxigenados y de carácter hidrofílico (Stevenson, 1994).

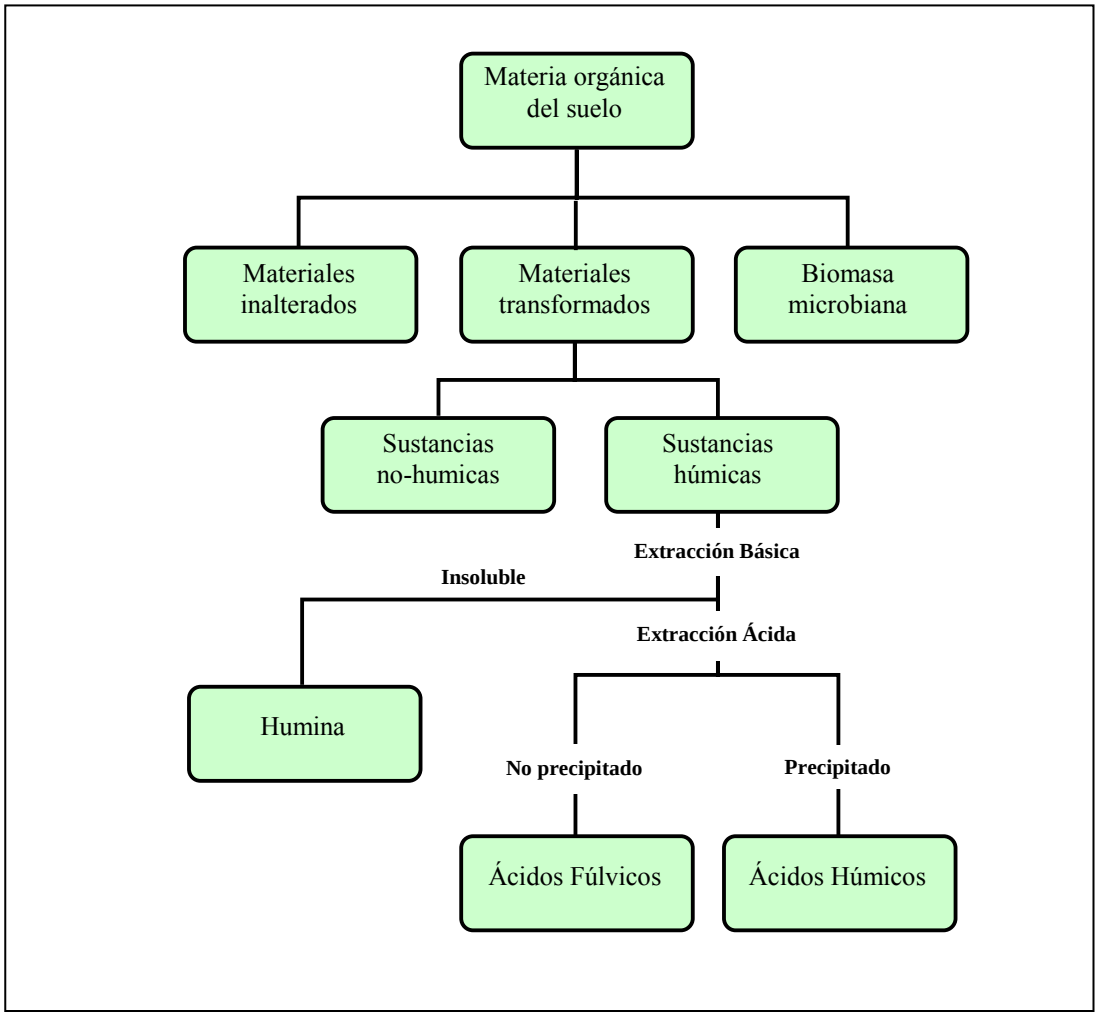

Figura I.2: Composición de la materia orgánica .

Si bien la problemática de la definición de una estructura para las sustancias húmicas ha sido afrontada por muchos investigadores que han propuesto diversos modelos,

actualmente el empleo de modernas técnicas analíticas como la RMN ^{13}C, la espectroscopía de fluorescencia o la resonancia de espín electrónico, entre otras, han proporcionado más información acerca de las complejas estructuras de estos compuestos (Schnitzer, 1991). La mayoría de los resultados obtenidos indican que estos materiales están constituidos, en gran medida, por anillos aromáticos unidos entre sí y a otras estructuras de carácter alifático. Sin embargo, a pesar de toda su complejidad y de lo difícil que resulta dar una estructura adecuada para definir las sustancias húmicas, su interés agronómico supera dichas complicaciones ya que no es imprescindible averiguarla de manera exacta para tener un buen conocimiento de la importante función de las mismas (Hayes, 1991).

I.3. LAS ENMIENDAS ORGÁNICAS Y LOS RESIDUOS

Como se ha comentado anteriormente, existe una estrecha relación, ampliamente constatada, entre la pérdida de materia orgánica, la merma del potencial agrícola y el avance de la erosión, en especial cuando confluyen otros factores como la ausencia de vegetación, la pendiente del terreno y las condiciones climáticas (Stoate et al., 2001). Hoy en día, el uso de enmiendas orgánicas para restaurar, mantener o mejorar los contenidos de materia orgánica del suelo y sus funciones físicas, químicas y biológicas es una de las prácticas más comunes, eficientes y baratas (Senesi et al., 1996). Sin embargo, las enmiendas orgánicas usadas tradicionalmente, como los estiércoles o las turbas, en la actualidad no son suficientes para cubrir la demanda existente, lo que hace preciso buscar otros tipos de materiales alternativos provenientes de muy distintas fuentes (Saviozzi et al., 1999); dentro de esta lista de nuevas enmiendas orgánicas destacan por su abundancia y disponibilidad los residuos orgánicos, ya sean provenientes de actividades agrícolas y forestales (restos de cultivo, purines, astillas, serrines, etc.), urbanas (residuos urbanos, lodos de depuradora) o industriales (alpechines, orujos, vinazas, etc.) (Senesi et al., 1996; García-Gil et al, 2000; Brebbia et al., 2004; Brunetti et al., 2005, Mandal et al., 2006). Además, el uso de estos residuos orgánicos, aparte de constituir una posibilidad de aumentar los niveles de materia orgánica del suelo mediante su reutilización como enmiendas orgánicas, es una posible solución medioambientalmente viable al problema que supone su almacenamiento en vertederos o la incineración de unos

residuos cuya producción diaria está en constante aumento, especialmente en las áreas urbanas densamente pobladas.

Sin embargo, se debe tener en cuenta que los residuos orgánicos plantean también una serie de inconvenientes y restricciones cuando se aplican al suelo. Como residuos, debido a su origen y composición, pueden ser causa de problemas muy diversos tales como la acumulación de metales pesados, la presencia de contaminantes orgánicos o aumentos en la salinidad del suelo (García et al., 1994a; Antolín et al, 2005); pero además, como compuestos orgánicos, también deben ser tenidos en cuenta otros factores muy importantes como la estabilidad o tasa de descomposición de la materia orgánica y la madurez o grado de descomposición de las sustancias orgánicas fitotóxicas (Wu et al., 2000; Wang et al., 2004).

La aplicación de enmiendas orgánicas inestables y/o poco maduras puede inducir múltiples efectos adversos en las propiedades del suelo así como en el crecimiento de las plantas, incluyendo el aumento de la tasa de mineralización de la materia orgánica del suelo por un aumento de la actividad microbiana, la alteración del pH, la inmovilización del nitrógeno disponible provocando deficiencias en los cultivos, la introducción de compuestos fitotóxicos y de patógenos en suelo y agua, y la producción de amonio a unos niveles que puedan resultar tóxicos para la planta y contaminar el agua y/o el aire. Además, la descomposición en el suelo de enmiendas poco estables resta oxígeno a la respiración radicular y a los procesos de nitrificación lo que conlleva la formación de compuestos fitotóxicos como nitritos y sulfitos. Así mismo, una materia orgánica inmadura y/o inestable puede contener una proporción alta de compuestos orgánicos en formas hidrosolubles, los cuales pueden ser transportados mediante procesos de lixiviación y lavado de los suelos a aguas superficiales y subterráneas afectando a su calidad (Mathur et al., 1993; Senesi et al., 1996).

Por todo ello, con el fin de hacer un uso correcto de los residuos orgánicos en agricultura, pero con un riesgo limitado para el medioambiente, es necesario que los residuos sean previamente tratados con el fin de obtener enmiendas medioambientalmente seguras y agronómicamente eficaces así como evaluar la estabilidad y la madurez de estos materiales y su validez como producto final antes de ser aplicados al suelo (Williams, 2005).

I.4. LOS LODOS DE DEPURADORA URBANAS

I.4.1. Origen

Una planta depuradora de aguas residuales consta fundamentalmente de dos grandes líneas de tratamiento: la correspondiente a la depuración de aguas y la del tratamiento de los lodos que se generan en las operaciones de decantación; esta última constituye una parte fundamental de las estaciones de depuración, donde se genera un subproducto que recibe la denominación de lodos de depuradora que se definen como "material sólido heterogéneo cuya composición no sólo depende del agua residual, sino también de la tecnología empleada y de la época del año" (Terry et al, 1979); el tipo de vertidos que confluyen en la depuradora, así como el proceso que experimentan los lodos en ésta y los hábitos sociales, determinarán la composición de este material orgánico.

I.4.2. Composición

La composición de los lodos es variable dependiendo de la calidad de las aguas y los vertidos, además de los tratamientos que hayan recibido; normalmente, están integrados por el conjunto de elementos que componen el efluente, junto con los aditivos químicos empleados en el tratamiento y la masa bacteriana que participa en el proceso de depuración. Además, contienen un porcentaje elevado en agua (50-80%), lo cual constituye una desventaja para su transporte, aunque en la actualidad se están implantando nuevas tecnologías de secado que disminuyen considerablemente estos contenidos.

Por sus características son un recurso importante de materia orgánica y de elementos fertilizantes, lo que les hace ser una fuente potencial de nutrientes para su utilización agrícola (**Tabla I.2**). Aproximadamente, el 50% de su peso en seco corresponde a materia orgánica, aportando además cantidades variables de nitrógeno (1-7%), fósforo (1-5%), potasio (0.3-3%) y micronutrientes con distinto grado de disponibilidad para las plantas (Soler et al., 1997).

De la calidad final de los lodos generados van a depender sus posibles destinos. La aplicación de tecnologías específicas de depuración para cada caso, además de una buena gestión de las plantas depuradoras y del control de los vertidos industriales, va a contribuir a la obtención de un material orgánico de mayor calidad y con riesgos menores para el medioambiente.

Tabla I.2. Composición y contenidos en los lodos de depuración de aguas urbanas (sobre materia seca).

	Contenido	Características agrícolas
Agua	50-80%	Condiciona su manejo y transporte
Materia orgánica	50%	Humificación similar a la natural
Nitrógeno	1-7%	Sobre todo orgánico, mineralizable
Fósforo	1-5%	Bastante disponibles
Potasio	0.3-3%	Bajo contenido pero soluble
Macroelementos	Variable	Según tratamientos, formas inorgánicas
Metales pesados	Variable	Puede ser un factor limitante
Microorganismos	Variable	Gran cantidad y variedad

Fuente: Soler, 1998.

I.4.3. Problemática actual

En la actualidad, la gestión de los residuos orgánicos urbanos se ha convertido en una de las prioridades dentro de las iniciativas destinadas a la protección del medioambiente y de la salud humana. Cada día se producen cantidades ingentes de residuos, de los cuales un porcentaje alto corresponde a los denominados lodos de depuradora. La problemática de estos residuos reside en los elevados volúmenes de producción que se generan, sin olvidar las consecuencias medioambientales y sanitarias que conlleva su inadecuada gestión; además, una vez extraídos de la planta de depuración los lodos deben someterse a un tratamiento basado en la estabilización del material mediante la activación de la descomposición de la materia orgánica y distintas operaciones de espesamiento, acondicionamiento y deshidratación para facilitar su manejo, transporte y almacenamiento.

La tendencia clara en los próximos años es hacia un aumento de la producción de los lodos de depuradora, consecuencia directa de la aplicación de la Directiva Comunitaria (91/271/CEE) relativa a las aguas residuales urbanas, unido a la ejecución del Plan Nacional de Saneamiento y Depuración de las aguas, que se traduce en España en el crecimiento de la construcción de estaciones depuradoras. Teniendo en cuenta las depuradoras actuales y futuras, su capacidad y la población a la que sirven, las estimaciones sitúan la producción de lodos en España en una cifra algo menor a las 1100 toneladas de residuo seco en el año 2005 (**Tabla I.3**).

Tabla I.3. **Estimaciones de generación, uso y gestión de lodos de depuradora dentro del Plan Nacional de Lodos de Depuradoras (2001-2006).**

	Total Unión Europea				**Total España**			
	2000		2005		2000		2005	
	t (m.s.)	(%)	t (m.s.)	(%)	t (m.s.)	(%)	t (m.s.)	(%)
Producción	7890	100	8331	100	1069	100	1088	100
Reciclado	4183	53	4536	54	578	54	589	54
Vertedero	1739	22	1554	19	360	34	367	34
Vertido al mar	57	1	57	1	57	5	57	5
Incineración	1731	22	1986	24	74	7	75	7
Otros	180	2	198	2	0	0	0	0

Fuente: DGXI, UE. BOE 166 (2001), expresado en miles de toneladas de materia seca.

En la actualidad, las alternativas de tratamiento de los lodos de depuradora son básicamente tres: depósito en vertedero, valorización energética o su utilización en agricultura. Sin embargo, para su aplicación agrícola es necesario un postratamiento de los mismos para mejorar sus propiedades y garantizar unas condiciones higiénico-sanitarias óptimas para su utilización. Los procesos tecnológicas existentes en la actualidad son múltiples y variadas, pero se pueden destacar fundamentalmente dos: uno de fiabilidad ampliamente contrastada como el "compostaje" y otro de reciente introducción y prometedores resultados como el "secado térmico".

I.4.4. Compostaje

Una vía clásica de tratamiento de los lodos de depuradora para convertirlos en productos aceptables para la utilización en el sector agrícola es el compostaje (Costa et al.,1991). Mediante este proceso se consigue estabilizarlos a través de una descomposición parcial de su materia orgánica, convertir parte de los elementos nutrientes en formas asimilables, destruir los microorganismos patógenos y eliminar malos olores y sustancias fitotóxicas.

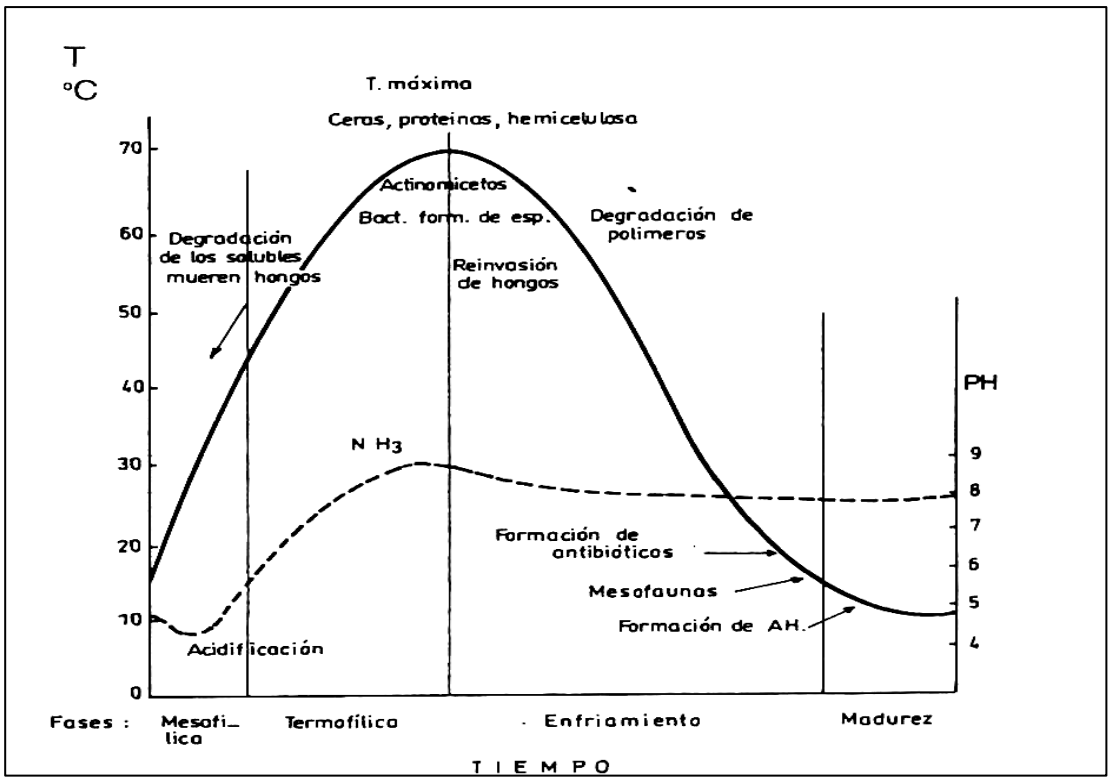

Figura I.3: Evolución de la temperatura y pH durante las diferentes etapas del compostaje (Dalzell et al., 1981)

El compostaje se define como "un proceso bioxidativo controlado, en el que intervienen numerosos y variados microorganismos, que requiere una humedad adecuada y substratos orgánicos heterogéneos en estado sólido, implica el paso por una etapa termófila y una producción temporal de fitotoxinas, dando al final como productos de los procesos de

degradación: agua, dióxido de carbono y una materia orgánica estabilizada, libre de sustancias fitotóxicas y dispuesta para ser aplicada en la agricultura" (Senesi, 1989; Francis, 1998) (**Figura I.3**.).

Existen dos grandes categorías de sistemas para la realización del compostaje: los abiertos donde los residuos se colocan en grandes pilas estáticas o asociadas a volteos con ventilación forzada o no (**Figura I.4**) y los cerrados donde se utilizan reactores o digestores.

Figura I.4. Ejemplo de un sistema de compostaje abierto y con volteos.

El compostaje precisa un control exhaustivo para su buen desarrollo y para conseguir un producto final que sea aceptable y apto para su utilización como abono orgánico. Los parámetros que se deben controlar son: microorganismos, humedad, temperatura, aireación, tamaño de partícula, pH y relación C/N. El material obtenido, denominado compost, deberá ser un producto inocuo para su aplicación en la agricultura. Así, se define como compost maduro aquel que se puede aplicar al suelo sin riesgos de fitotoxicidad, tiene una materia orgánica estable y su contenido en elementos nutritivos para la planta es óptimo.

Sin embargo, a pesar de los buenos resultados obtenidos, el compostaje plantea algunos problemas como son sus altos requerimientos de espacio y tiempo así como otros

derivados de la maduración de la materia orgánica como pueden ser los malos olores, lo que aconseja que se desarrollen otros procedimientos alternativos.

I.4.5. Secado térmico

Un tipo de tratamiento que ha irrumpido con fuerza en nuestro país en los últimos años es el secado térmico, que consiste en la eliminación del agua que contienen estos residuos mediante su evaporación. La transmisión de calor necesario para lograr dicha evaporación se efectúa por medio de un fluido térmico (aire, vapor o aceite), que es el que aporta el calor necesario al proceso.

Gracias a este postratamiento se consigue un porcentaje muy alto de materia seca del orden del 90% (que supera al 65% obtenido mediante el compostaje). Además, las altas temperaturas a las que son sometidos los lodos durante el proceso de secado, en combinación con los periodos de permanencia en el interior del secador, aseguran la eliminación o inactivación de los microorganismos patógenos que inicialmente pudieran contener (Galdós, 2001).

En la **Figura I.5** se representa el esquema de secado térmico basado un proceso de convección indirecta asociado a un sistema de cogeneración de energía mediante un turbogenerador. Las fases de la línea de secado son las siguientes:

1. Transporte y almacenamiento de los lodos húmedos en los fosos de almacenamiento que pasan posteriormente a las tolvas dosificadoras.

2. Los lodos húmedos se mezclan en una mezcladora en continua de paletas con el material ya seco para conseguir un producto con un porcentaje de materia seca del 60%. La mezcla se introduce en un trómel (tambor rotativo) a una temperatura de 50 ºC mediante un tornillo transportador.

3. El lodo se seca con aire caliente a 320 ºC en el tambor rotativo. El producto de salida tiene el 90% de materia seca y una temperatura inferior a 75 ºC; el tiempo de permanencia en el tambor de secado es aproximadamente de 20 minutos a una temperatura de 70 ºC, produciéndose, la pasteurización del producto.

4. Los gránulos secos se separan del aire de secado en un cajón decantador y se criban de forma que la fracción con un diámetro entre 2 y 4 mm se enfría en un tornillo enfriador y se almacena (**Figura I.6**). El resto se recircula para su posterior mezclado con los lodos húmedos.

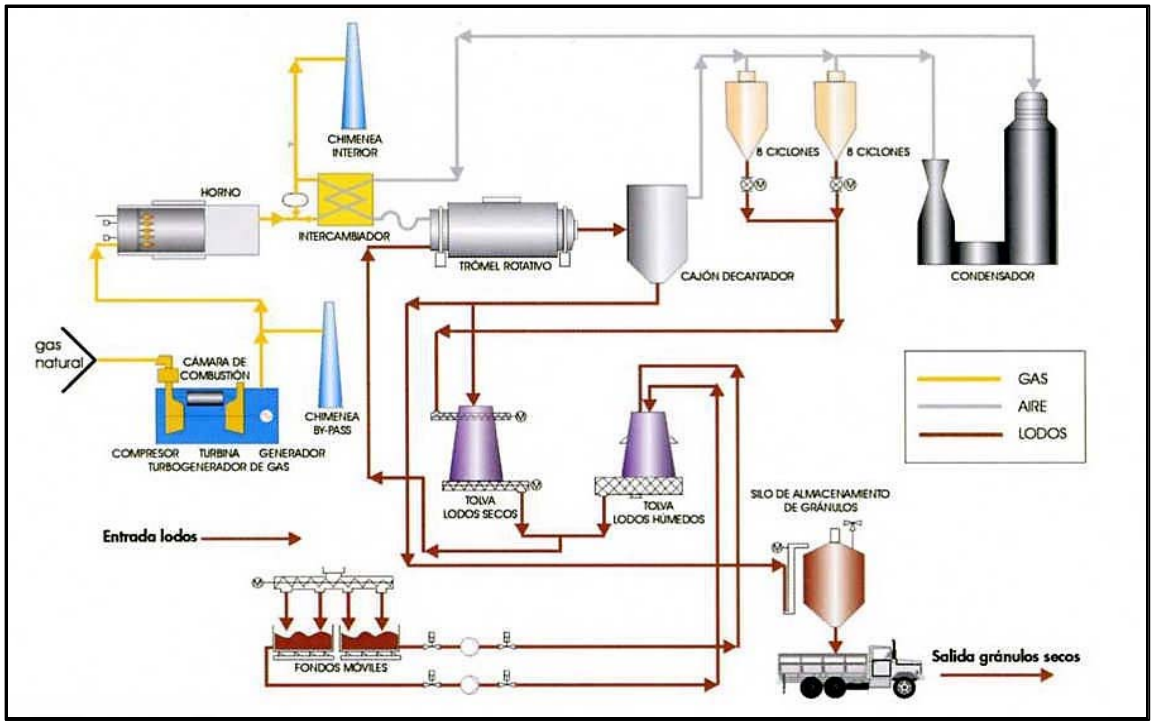

Figura 1.5. Diagrama general del proceso de convección indirecta del secado térmico.

La estabilidad del producto final es uno de los aspectos más complicados de precisar; el calor permite una cierta estabilización del lodo pero no provoca una reducción apreciable de la materia orgánica presente. Los lodos secados térmicamente no son estables, a menos que hayan sido digerido previamente; por tanto, existe la posibilidad de que el producto final se reactive en condiciones de humedad y temperaturas adecuadas.

Figura I.6. Producto final del proceso de secado térmico.

I.5. EFECTOS DE LOS LODOS SOBRE SUELOS Y PLANTAS

I.5.1. Efectos sobre la materia orgánica del suelo

Como se ha comentado anteriormente, uno de los objetivos fundamentales que se persigue con la aplicación de residuos orgánicos al suelo es incrementar su contenido de materia orgánica. La incorporación de lodos de depuradora, especialmente ricos en carbono orgánico, puede suponer un aporte apreciable, sobre todo en suelos de zonas semiaridas, que presentan niveles bajos de materia orgánica.

Las fracciones de carbono con distintos grados de estabilidad pueden experimentar varios procesos en el sistema tras su incorporación al suelo, incluyendo la mineralización, la asimilación por la biomasa microbiana o la incorporación a la fracción húmica estable del mismo. En la dinámica de estos procesos influyen, además de las propiedades de los lodos, las características y el manejo que recibe el suelo, así como factores físicos, químicos, biológicos y ambientales (Batjes, 1998). En general, los ácidos húmicos de los suelos enmendados tienen características intermedias en su composición elemental y contenidos en grupos funcionales entre los suelos sin enmendar y los aportados con las enmiendas

orgánicas (Brunetti et al., 1994); sin embargo, estos efectos se hacen menos aparentes con el trascurso del tiempo (García-Gil et al., 2004).

I.5.2. Efectos sobre las propiedades físico-químicas del suelo

La incorporación de lodos de depuradora a los suelos produce cambios en el pH y en la conductividad eléctrica, en un orden de magnitud que varía según las características de cada suelo, de la dosis y frecuencia de aplicación de estos materiales (Nogales, 1994), así como de las condiciones climáticas de la zona.

El pH de los suelos va a condicionar la disponibilidad de nutrientes y la movilidad de determinados metales pesados, así como la actividad microbiana del sistema. Los lodos de depuradoras normalmente tienen un pH próximo a la neutralidad; en suelos ácidos o ligeramente ácidos, la adición de estas enmiendas produce un aumento del pH (He et al., 1992; Díaz-Marcote y Polo, 1996), mientras que en los neutros o alcalinos los resultados son menos homogéneos, no constatándose variaciones incluso a dosis altas (Hernández et al., 1992, Balanyá et al., 1994), aunque algunos autores han observado descensos en suelos ligeramente básicos (Climent et al., 1996; Soler et al., 1998).

Los lodos de depuradoras producen un aumento del contenido en sales y de la conductividad eléctrica en los suelos (Allué et al., 1994; Climent et al., 1996), sobre todo a dosis elevadas, lo cual puede constituir una limitación desde el punto de vista de su uso agrícola, al poder elevarse los valores por encima de los tolerables por los cultivos (Gallardo-Lara y Nogales, 1987), especialmente en los agrosistemas mediterráneos donde el régimen de precipitaciones es escaso y el lavado de las sales en el perfil del suelo es limitado. No obstante, algunos autores han observado un descenso en el contenido en sales de los horizontes superficiales del suelos cuando se aplican dosis bajas de estos residuos, efecto atribuido a la mejora de las condiciones físicas del sistema, favoreciendo la infiltración del agua y el lavado de sales (Canet et al., 1996).

I.5.3. Efectos sobre la disponibilidad de nutrientes en el suelo

Los residuos orgánicos urbanos aumentan los contenidos en nutrientes del suelo, debido a que presentan cantidades importantes en su composición (Senesi, 1989), especialmente de nitrógeno y fósforo en el caso de los lodos de depuradora (Díaz-Burgos y Polo, 1991).

La eficiencia de estos materiales como fertilizantes depende de diversos factores, entre los que está el tipo de suelo y de cultivo empleado, la calidad del lodo y los factores ambientales que van a incidir directamente sobre los procesos de mineralización y la disponibilidad de nutrientes para la planta (Gallardo-Lara y Nogales, 1987). Por estos motivos, su aplicación debe realizarse en función de los requerimientos nutricionales del cultivo, tanto en el tiempo como en la dosis, con el fin de minimizar las pérdidas por lixiviación, en especial de nitratos y fosfatos, evitando fenómenos contaminantes sobre los acuíferos.

La mineralización del nitrógeno orgánico contenido en estas enmiendas está determinada tanto por factores físico-químicos como biológicos, por lo que la estimación de la carga de nitrógeno que se debe aplicar al suelo para una correcta fertilización del cultivo debe considerar los procesos a los que está sometido este elemento en el suelo. La disponibilidad para la planta del fósforo es buena y se puede equiparar a la ofrecida por los fertilizantes minerales fosforados (Ayuso et al., 1992). Los lodos presentan valores bajos de potasio, aunque su disponibilidad para los cultivos es inmediata, mientras que los contenidos de calcio y magnesio son muy variables (Soler, 1998).

I.5.4. Efectos sobre las propiedades biológicas y bioquímicas del suelo

La incorporación de residuos orgánicos a los suelos va a producir una reactivación de sus propiedades biológicas y bioquímicas, estimulando la proliferación microbiana y su actividad metabólica, como consecuencia de los aportes de nuevas fuentes lábiles de carbono que van a servir como sustrato a la biota del suelo. La estimulación de la actividad

microbiana en los suelos va acompañada de un incremento de su tasa respiratoria, desprendiéndose dióxido de carbono como reflejo de los procesos catabólicos llevados a cabo sobre las fracciones orgánicas añadidas. Además, la revitalización del sistema se traduce en un incremento de las actividades enzimas en el suelo, implicadas en los distintos ciclos biogeoquímicos de descomposición e inmovilización de los nutrientes incorporados con los materiales orgánicos (Gil-Sotres et al., 1992).

El estudio de algunos parámetros biológicos de los suelos, como la biomasa microbiana y su actividad respiratoria, junto con las estimaciones de sus actividades enzimáticas oxidorreductasas e hidrolasas, pueden servir como bioindicadores sensibles a los cambios que están ocurriendo sobre la materia orgánica (Ceccanti y García, 1994; Bandick y Dick, 1999) y sobre el estado ecológico de los suelos.

I.5.5. Efectos sobre los cultivos

Los efectos que sobre la producción tienen la adición de estos residuos orgánicos, presentan gran variabilidad, dependiendo de la calidad, de la dosis aplicada, del tipo de cultivo y de las condiciones edafoclimáticas principalmente, aunque en general, pueden considerarse positivas cuando se emplean en dosis moderadas (Gallardo-Lara y Nogales, 1987).

La utilización de lodos de depuradora como enmienda orgánica y fuente de nutrientes para diversos cultivos agrícolas ha sido estudiada por distintos autores (Ingelmo et al., 1998; Antolín et al., 2005). En el cultivo de cebada en condiciones de secano también ha habido estudios previos que detallan las respuestas de esta gramínea ante distintos manejos y tratamientos orgánicos con lodos (Moreno et al., 1996; Soler et al., 1998).

En general, la eficiencia como fertilizantes de estas enmiendas es menor que la ofrecida por los agroquímicos convencionales (Ryan et al., 1985), debido principalmente a los procesos de mineralización que se tienen que llevar a cabo para la disponibilidad de algunos nutrientes que se encuentran en formas orgánicas. Sin embargo, las sustancias

húmicas que se incorporadas con estas enmiendas al suelo, ejercen múltiples efectos sobre los procesos bioquímicos y fisiológicos del crecimiento de las plantas, favoreciendo su germinación (Lulakis y Petsas, 1995), desarrollo vegetativo (Chen et al., 1994) y radicular (Chen y Aviad, 1990). Estos compuestos poseen además una capacidad elevada para formar complejos y quelatos relativamente estables, con cationes polivalentes (Cu^{2+}, Mn^{2+}, Fe^{3+}, etc.) que facilita la posibilidad de ser asimilados por las plantas (Costa et al., 1991; Lucena, 1990). Todos estos efectos contribuyen a una absorción mejor de los nutrientes por el cultivo, repercutiendo positivamente sobre su rendimiento final.

I.5.6. Problemática de los metales pesados

Como se ha comentado anteriormente, la aplicación de enmiendas orgánicas conlleva una serie de riesgos e inconvenientes que deben ser tenidos en cuenta, y entre ellos, el contenido en metales pesados es probablemente el factor que más limita la utilización de los lodos de depuradora en agricultura.

Dado que la composición de estos residuos es bastante heterogénea, en función del origen y de los tratamientos previos que reciban, sus contenidos en metales pesados serán muy variables. La concentración de estos elementos en la solución del suelo está controlada por una serie de procesos interrelacionados, entre los que destacan la formación de complejos de naturaleza orgánica e inorgánica, reacciones de oxidación-reducción, de precipitación-disolución y de adsorción-desorción. Al mismo tiempo, los metales en la disolución del suelo están sometidos a posibles pérdidas por lixiviación, absorción por las plantas o volatilización, siendo este último un mecanismo importante para elementos como el mercurio, selenio y arsénico (Cala, 1998).

La tendencia en los últimos años es a disminuir los contenidos en metales pesados en los lodos de depuradora con la entrada en vigor de las normativas que regulan la depuración de las aguas residuales y la gestión de lodos. Esta legislación limita su uso agrícola en función de los niveles de metales pesados existentes.

I.6. NORMATIVA LEGAL

El Plan Nacional de Lodos de Depuradoras establece directrices para minimizar las prácticas de eliminación por incineración y vertederos controlados e incrementar la reutilización y el reciclado de estos residuos con fines agrícolas o para la conservación de suelos (**Tabla I.4**).

Tabla I.4. **Gestión y usos previstos de los lodos de depuradora a finales del año 2006**

	toneladas (m.s) año $^{-1}$	**%**
Uso agrícola y conservación de suelos (No compostados)	555244 - 619190	40
Uso agrícola y conservación de suelos (Compostados)	345778 - 386994	25
Incineración (con recuperación de energía)	276622 - 309595	20
Depósito en vertederos	205470 - 232196	15
TOTAL	1383114 - 1547976	100

Fuente: MIMAM. BOE 166 (2001).

Sin embargo, el empleo de estos residuos orgánicos en agricultura puede ocasionar problemas debido a que pueden incorporar sustancias peligrosas, especialmente aquellos lodos procedentes de las zonas industriales. Los factores que puede limitar su utilización agronómica son: exceso de salinidad, exceso de nutrientes, contaminantes orgánicos, microorganismos patógenos y metales pesados.

Actualmente la Directiva 86/278/CEE de 12 de Junio, relativa a la protección del medioambiente y de los suelos en particular, regula la utilización de lodos de depuradora en agricultura, establece los estándares de calidad mínimos y define los requisitos de control. La Unión Europea está trabajando en la revisión de esta Directiva a fin de adaptarla al progreso técnico y científico y armonizarla con otros referentes legales más recientes como la Directiva marco de Residuos (91/156/CEE) que ratifica su gestión y con la Directiva sobre Vertido (1999/31/CEE) que restringe la posibilidad de eliminación en vertederos de residuos biodegradables y prohíbe el vertido de los que no se hayan sometido a tratamiento.

La Directiva 86/278/CEE fue transpuesta al Ordenamiento Jurídico Español mediante el Real Decreto 1310/1990, de 29 de octubre (BOE 262, 1990), desarrollado por la Orden Ministerial de 26 de octubre de 1993 (BOE 265, 1993). En la **Tabla I.5** se recogen los contenidos máximos de metales pesados en suelos y en lodos de depuradora admitidos por la legislación vigente, si bien todavía existe un vacío legal que regule los contenidos máximos de compuestos orgánicos tales como hidrocarburos aromáticos policíclicos, bifenilos policlorados, fenoles y otros químicos de naturaleza tóxica que están presentes en los lodos de depuradoras.

Tabla I.5. Valores límite para metales pesados en suelos y en lodos de depuradora para su aplicación agrícola (Real Decreto 1310/1990)

	Valores límite en suelos (mg kg^{-1})		**Valores límites en lodos (mg kg^{-1})**		**Cantidades anuales (1)**
	Suelos pH<7	**Suelos pH>7**	**Suelos pH<7**	**Suelos pH>7**	
Cadmio	1	3	20	40	0.15
Cobre	50	210	1000	1750	12.00
Níquel	30	112	300	400	3.00
Plomo	50	300	750	1200	15.00
Zinc	150	450	2500	4000	30.00
Mercurio	1	2	16	25	0.10
Cromo	100	150	1000	1500	3.00

(1) Valores límites para cantidades anuales de metales pesados que se podrán introducir en los suelos basándose en una media de diez años (kg ha^{-1} $año^{-1}$)

La Directiva 86/278/CEE de 12 de Junio referente a la protección de los suelos, está estrechamente relacionada con la Directiva 91/676/CE de 12 de Diciembre, relativa a la protección de las aguas contra la contaminación producida por nitratos de origen agrario, transpuesta al Ordenamiento Jurídico Español mediante Real Decreto 261/1996, de 16 de febrero (BOE 61, 1996). Dicha directiva obliga a los Estados miembros a establecer las zonas vulnerables a dicha contaminación y Códigos de Buenas Prácticas Agrarias como marco de referencia para el desarrollo de una agricultura compatible con el medioambiente, lo que supone la exigencia de una aportación racional de nitrógeno a los cultivos.

En lo que respecta a la elaboración del Plan Nacional de Lodos de Depuradoras de Aguas Residuales, el Ministerio de Medio Ambiente contempla para el período comprendido entre los años 2001-2006, la aplicación correcta de las Directivas mencionadas, así como la consecución de los siguientes objetivos ecológicos (BOE 166, 2001):

- Reducción en origen de la contaminación de los lodos.
- Caracterización de los mismos.
- Valoración agronómica y energética para reducir su eliminación mediante vertederos.
- Creación de un sistema estadístico y bases de datos sobre lodos y su gestión.

En resumen, la aplicación de lodos de depuradora en agricultura es una salida recomendable, siempre que se efectúe en condiciones que garanticen la protección del suelo y de las aguas superficiales y subterráneas. Por ello, resulta obligado controlar tanto las cantidades incorporadas como su efecto acumulativo. Es necesaria una caracterización previa de los lodos y de los suelos donde se van aplicar para comprobar que cumplen la normativa vigente y sobre todo, para evitar posibles problemas de contaminación en el medioambiente o daños para la salud humana. Así mismo, es preciso hacer un seguimiento del efecto que estos residuos ejercen sobre el sistema suelo-planta mediante los análisis que permitan conocer de forma concreta la evolución de los distintos parámetros que definan la fertilidad de los suelos y las características de los cultivos.

II. OBJETIVOS

II. OBJETIVOS

El suelo es un recurso de gran importancia y complejidad que presenta gran heterogeneidad en sus propiedades. En la región mediterránea, las condiciones climáticas, unidas a un manejo inadecuado del suelo por las practicas agrarias, han favorecido la pérdida de materia orgánica acelerando los procesos degradativos de los suelos, disminuyendo el potencial agrícola de los mismos e incrementando los riesgos de erosión y desertificación.

El abonado orgánico tradicional no es suficiente para paliar estas necesidades de materia orgánica por lo que es necesario la búsqueda de nuevos materiales orgánicos de bajo coste que sean adecuados para este fin y se produzcan en cantidades suficientes. En este sentido los lodos de depuradoras urbanas, convenientemente tratados, constituyen una importante alternativa como fuente de materia orgánica. La realización de estudios de campo de larga duración proporciona una valiosa información sobre la capacidad fertilizante de estos residuos y la influencia que ejercen sobre el suelo y las plantas en condiciones reales.

El objetivo principal de este trabajo es "estudiar y comparar los efectos de la aplicación de dos clases de lodos de depuradoras urbanas, compostados y secados térmicamente, sobre un cultivo de cebada y sobre las propiedades físico-químicas, biológicas y bioquímicas de un suelo representativo de un agroecosistema semiárido, prestando especial atención a las transformaciones de la materia orgánica".

Con ello se pretende valorar la capacidad de estos lodos para restaurar la fertilidad y calidad del suelo y proveer al mismo tiempo una alternativa de bajo riesgo medioambiental para los actuales métodos de almacenamiento de estos residuos. El objetivo principal se puede desglosar en los siguientes **objetivos secundarios**:

1. Estudiar la mineralización de la materia orgánica aportada por estos residuos, examinado su evolución en el suelo bajo condiciones controladas de humedad y temperatura en laboratorio.

2. Determinar el efecto acumulativo y residual durante tres años de la aplicación de ambos lodos de depuradora sobre la dinámica de las fracciones del carbono orgánico del suelo profundizando en su influencia sobre la composición, características ácido-base y propiedades estructurales de las sustancias húmicas, con especial atención a las de los ácidos húmicos.

3. Evaluar la influencia de estos residuos en condiciones de campo sobre la actividad microbiana del suelo, implicada directamente en los ciclos biogeoquímicos de diferentes elementos, mediante la determinación de parámetros biológicos y bioquímicos que están considerados como indicadores sensibles a los procesos de regeneración o contaminación de los suelos.

4. Determinar la capacidad fertilizante de ambos lodos de depuradora estudiando sus efectos acumulativos y residuales a lo largo de tres años, sobre el rendimiento y calidad de un cultivo de cebada en condiciones de secano.

5. Valorar comparativamente el resultado final del proceso de secado térmico de los lodos frente al compostaje de los mismos.

III. CARBON MINERALIZATION IN AN ARID SOIL AMENDED WITH THERMALLY-DRIED AND COMPOSTED SEWAGE SLUDGES

III. CARBON MINERALIZATION IN AN ARID SOIL AMENDED WITH THERMALLY-DRIED AND COMPOSTED SEWAGE SLUDGES

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INDEX

ABSTRACT

Soil amendment with sewage sludge (SS) from municipal wastewater treatment plants is nowadays a common practice for both increasing soil organic matter and nutrient contents and waste disposal. However, the application of organic amendments that are not sufficiently mature and stable may adversely affect soil properties. Composting and thermal drying are treatments designed to minimize these possible deleterious effects and to facilitate the use of SS as a soil organic amendment. In this work, an arid soil either unamended or amended with composted sewage sludge (CS) or thermally-dried sewage sludge (TS) was moistened to an equivalent of 60% soil water holding capacity and incubated for 60 days at 28 ºC. The C-CO_2 emission from the samples was periodically measured in order to study C mineralization kinetics and evaluate the use of these SS as organic amendments. In all cases, C mineralization decreased after the first day. TS-amended soil showed significantly higher mineralization rates than unamended and CS-amended soils during the incubation period. The data of cumulative C-CO_2 released from unamended and SS-amended soils were fitted to six different kinetic models. A two simultaneous reactions model, which considers two organic pools with different degree of biodegradability, was found to be the most appropriate to describe C mineralization kinetics for all the soils. The parameters derived from this model suggested a larger presence of easily biodegradable compounds in TS-amended soil than in CS-amended soil, which in turn presented a C mineralization pattern very similar to that of the unamended soil. Furthermore, net mineralization coefficient and complementary mineralization coefficient were calculated from C mineralization data. The largest losses of C were measured for TS-amended soil probably due to an extended microbial activity. The results obtained thus indicated that CS is more efficient for increasing total organic C in arid soils.

Keywords: Sewage sludge; Composting; Thermal drying; Carbon mineralization; Model fitting; Organic amendment.

III.1. INTRODUCTION

During the last years the amount of sewage sludge (SS) from urban wastewater treatment plants has increased steadily with consequent environmental and economic problems related to its disposal (Albiach, 2001). The high organic matter (OM) content of SS has favoured a growing interest in its use as a soil organic amendment, especially in arid and semi-arid Mediterranean areas. The soil organic matter (SOM) content in these areas is typically low and tends to decrease for different reasons like inappropriate cultivation practices, erosion or climatic conditions (Pascual et al., 1999; García-Gil et al., 2000).

However, before land application, SS should be subjected to appropriate treatments, designed to enhance the stability of OM and to avoid a number of harmful effects on soil properties as well as many health hazards that may result from an inadequate use (Senesi and Brunetti, 1996; Plaza et al., 2003). One common treatment for this purpose is composting, a biological process of aerobic decomposition that degrades labile organic matter to water vapour, CO_2, ammonia, inorganic nutrients and stable organic material containing humic-like substances (Senesi, 1989). Composting allows to obtain an environmentally-safe and agronomically-advantageous soil organic amendment, but has important requirements of time (over three months) and space (for the composting piles). An alternative and relatively newer process is the so called "thermal drying" by which SS is treated by intense heating (over 300 ºC). Thermal drying inactivates pathogens and volatile chemicals and leads to a sanitized final product in pellets in relatively short time, with low odours and good handling characteristics but without maturation process.

From an environmental and agronomical point of view, a correct management of the SS in agriculture relies mainly on two aspects: efficient increase of the SOM and adequate match of the release of mineral nutrients to crop demand. Therefore, the knowledge of C mineralization dynamics in SS amended soils is of intrinsic interest. The effects of composted sewage sludge (CS) on soil properties and C mineralization have been the focus of intensive research, but only a limited number of investigations have dealt with the effects of thermally-dried sewage sludge (TS), and even fewer studies have been conducted on C

mineralization in soils amended with TS. Thermally-dried sewage sludge is richer in N and soluble organic carbon (e.g., aminoacids and carbohydrates) than CS, two factors that can stimulate the soil microbial metabolism and consequently the mineralization of C.

Even if carried out over short time periods, the use of laboratory methods involving incubation of soil-waste mixtures under controlled conditions can supply accurate information about C mineralization dynamics. In addition, C mineralization data obtained can be fitted to kinetic models, which allow calculating the fraction of potentially mineralizable C and its mineralization rate and, therefore, the real benefits of applying these organic wastes to soil (Saviozzi et al., 1993; Bernal et al., 1998). The first-order model by Stanford and Smith (1972) is the most widely used, although many other models have been also postulated. These include linear (Levi-Minzi et al., 1990) and non-linear regression expressions (Smith et al., 1980; Talpaz et al., 1981; Boyle and Paul, 1989; Murwira et al., 1990, Campbell et al., 1991), and first-order equations with parameters related to groups of substrates of various degrees of stability (Molina et al., 1980; Jones, 1984; Lindemann and Cardenas, 1984; Murayama et al., 1990). These mathematical descriptions of C release patterns can provide useful indices and allow the testing of hypotheses concerning the involved mechanisms.

The aim of this work was to describe comparatively the dynamic and kinetics of C mineralization in an arid soil amended with either TS or CS and in the same soil unamended. These results can be used to evaluate the degree of maturity and stability achieved in both organic amendments and, therefore, their environmental safety and agronomic efficiency.

III.2. MATERIALS AND METHODS

III.2.1 Soil and sewage sludge

The CSS used in this work was collected from a mixture of three SS originated from three municipal wastewater treatment plants in Madrid (Spain) metropolitan area, which has been subjected to a windrow composting process for three months. The TS sample was

collected from the wastewater treatment plant SUR in Madrid metropolitan area where the SS is dried in a process that use hot air at temperatures ranging from 380ºC to 450ºC. The soil sample used in this experiment is characterized by a sandy loam texture (sand, 580 g kg^{-1}; silt, 240 g kg^{-1}; clay, 180 g kg^{-1}) and was taken from the arable layer (Ap horizon, 0-20 cm depth) of a Typic Haploxeralf (Soil Survey Staff, 2003), which is located in the experimental farm "La Higueruela" (Toledo, Spain). The main chemical properties of the CS, TS and soil are included in **Table III.1**.

Table III.1: Main chemical properties (±standard errors) of soil, composted sewage sludge (CS) and thermally-dried sewage sludge (TS) used in the experiment.

	Soil	CS	TS
pH (H_2O)	5.7±0.1	7.1±0.1	7.0±0.1
Electrical Conductivity (dS m^{-1})	0.05±0.01	3.90±0.01	1.50±0.02
Total Organic Carbon (g kg^{-1})	7.2±0.1	181.0±0.2	296.0±0.2
Total Extractable Carbon (g kg^{-1})	1.4±0.2	53.4±0.2	87.7±0.3
Humic Acids Carbon (g kg^{-1})	0.7±0.1	21.0±0.3	24.8±0.2
Fulvic Acids Carbon (g kg^{-1})	0.6±0.1	32.4±0.3	62.9±0.3
C/N	8.0±0.1	7.6±0.2	8.3±0.2
Total N (g kg^{-1})	0.9±0.1	23.9±0.1	35.6±0.1
P (g kg^{-1})	0.09±0.01[a]	13.90±0.02[b]	13.43±0.02[b]
K (g kg^{-1})	0.20±0.01[a]	5.02±0.07[b]	4.29±0.05[b]

[a] Available content

[b] Total content

2.2 Incubation experiment

Carbon mineralization was studied in an aerobic incubation experiment carried out in a closed system. A hundred grams of air-dried and 2-mm sieved sample of soil homogeneously mixed with CS or TS, according to the field rates of 80 Mg ha^{-1} (assuming a soil bulk density of 1.5 g mL^{-1} for the top 20 cm), were placed in 500 mL hermetically sealed flasks . Also 100 g of soil without any amendment were run as control. Water was added to an equivalent of 70% of the soil water holding capacity and flasks were introduced in a thermostated bath at 28ºC for 60 days. There were four replicates for each treatment. The

quantity of C-CO_2 released was measured daily over the first eleven days and thereafter at days 13, 15, 17, 20, 23, 26, 29, 34, 39, 44, 48, 54 and 60. For this purpose a forced stream of CO_2-free-air was circulated during 2 hours through the flasks and then bubbled into a solution of 0.1 M NaOH, where CO_2 displaced from the flasks was trapped in. Then, excess alkali in this solution was back-titrated with standard 0.2 N HCl after precipitating carbonate with 1.5 M $BaCl_2$ solution (Polo et al., 1983).

III.2.3 Kinetic models

Six different models have been used in this experiment to describe the C mineralization in the samples studied.

The first model referred herein as zero-order (Seyfried and Rao, 1988) is expressed by the function:

$$C_t = kt + \text{intercept} \qquad (1)$$

where C_t is the cumulative organic carbon mineralized (mg C-CO_2 kg^{-1}) at time t (d), k (mg kg^{-1} d^{-1}) is a zero-order rate constant and the intercept represents a pool of highly mineralizable C.

The second model was used by Levi-Minzi et al., (1990) to describe net mineralization with an exponential kinetic:

$$C_t = kt^m \qquad (2)$$

where k and m are constants, k characterizes the units used for the variables (mg kg^{-1} d^{-1}) and m the shape of the curve.

Another approach is the first-order exponential equation (Murwira et al., 1990):

$$C_t=C_0(1-e^{-kt}) \qquad (3)$$

C_0 represents the amount of total potentially mineralizable C (mg kg^{-1}) and k is the mineralization rate constant (d^{-1}).

Molina et al. (1980) pointed out that this exponential equation does not account for the mineralization of large amounts of C during the initial weeks of incubation. These authors described the two simultaneous reactions model, another first order exponential equation, but with two components, two pools of mineralizable C, one that decomposes faster, during the first weeks of incubation, and another that decomposes more slowly:

$$C_t=C_1(1-e^{-k_1t})+C_2(1-e^{-k_2t}) \qquad (4)$$

where C_1 and C_2 represent the active and resistant pools decomposing at specific rates of k_1 and k_2. The sum of C_1 and C_2 has the same physical meaning as C_0 in the first order exponential model.

Another variation of the first order model is the first-order E model (Jones, 1984):

$$C_t=C_0(1-e^{-kt})+C_1 \qquad (5)$$

which uses an additional parameter C_1 (mg C kg^{-1}) that defines a separate pool of easily decomposable substrate that produces a mineralization flush during the first incubation interval. C_0 is the size of the active C fraction (mg C kg^{-1}) and k is the rate constant for the decomposition of the active C fraction (d^{-1}).

Finally the special model (Bonde and Roswal, 1987) is an approximation of the double exponential model for incubation experiments which duration is short in comparison with the half-life of the large resistant pool:

$$C_t=C_0(1-e^{-k_1t})+k_2t \qquad (6)$$

where C_0 and k_1 have the same meaning as C_0 and k in the first order model, and k_2t represents the large resistant C pool that mineralizes at a low and constant rate.

Model parameters for each treatment examined were calculated by non-linear regression analysis with the Quasi-Newton iterative method from cumulative C-CO_2 data. The coefficient of determination (R^2) was used for comparison of model fits.

III.2.4 Mineralization parameters

The net mineralization coefficient (NMC) and complementary mineralization coefficient (CMC) were calculated to assess the mineralizing capacity of soil in the presence of the SS and the susceptibility of TSS and CSS to mineralization (García et al., 1992). The NMC and CMC parameters are calculated as follows, based upon the cumulative amount of C evolved as CO_2 after 60 days:

$$NMC = C_A\ 100/(C_C + C_B) \quad (9)$$

$$CMC = (C_A\text{-}C_D)100/C_C \quad (10)$$

where C_A is the total C released as CO_2, C_B is the total organic C in the soil, C_C is the total organic C added with SS and C_D is the C emitted from the control as CO_2.

The Daily Complementary Mineralization Coefficient (DCMC) was also calculated as the CMC for each day of experiment. Besides the half-life time ($t_{1/2}$) of the different fractions of organic matter was calculated as $t_{1/2} = 0.693/k_n$.

Analytical data were subjected to one-way ANOVA test. Mean separation was performed with the least significant difference (LSD) test when F-test was significant at a 0.05 probability level.

III.3. RESULTS AND DISCUSSION

III.3.1 Carbon mineralization curves

During the experiment, daily release of C-CO_2 (**Figure III.1**) is larger for the soil amended with TS than for the control and CS amended soils, which yield similar values. For all the samples, the maximum C mineralization occurs the first day of incubation. Although secondary peaks appear afterwards, especially in the case of the soil amended with TS, a gradual decrease is observed for all samples. The amount of C-CO_2 released become fairly constant after the first 7 days for control and CS-amended soil while soil amended with TS reach a moderately constant trend after 28 days. The cumulative mineralized C (**Figure III.2**) for both unamended and CS-amended soils increases slightly with time and without significant differences. The increase is much more marked for soil amended with TS, especially during the first 28 days.

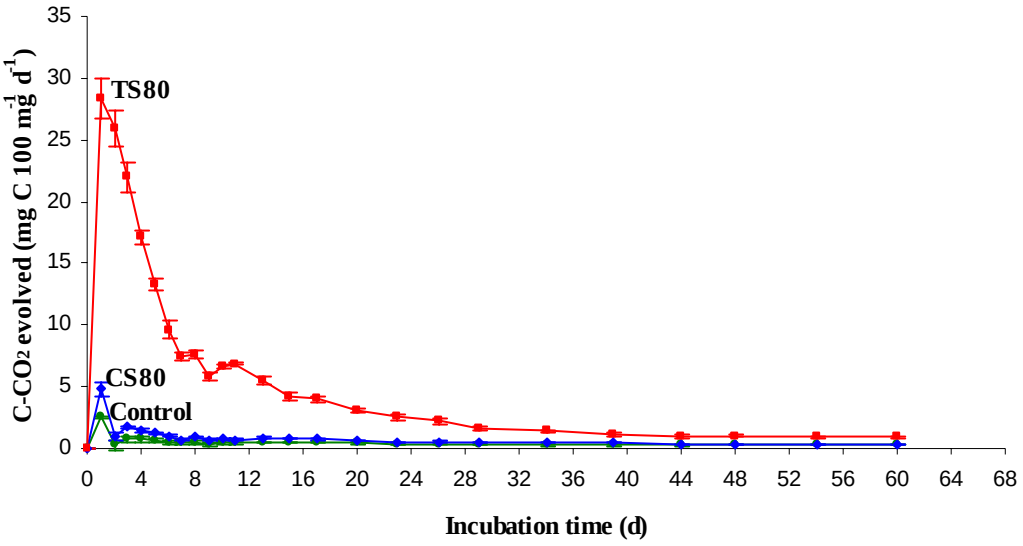

Figure III.1. Daily C released as CO_2 from unamended soil (Control) and soil amended with composted sewage sludge (CS80) or thermally-dried sewage sludge (TS80) during incubation. The bars indicate standard errors

The maximum C-CO_2 emission was measured at the first day of incubation, probably due to the favourable conditions of humidity and temperature (Tester and Parr, 1993; Pascual et al., 1998). The existence of secondary maxima may be attributed to the great variety of

compounds contained in the samples and their different degrees of degradability (Bernal et al., 1998). Besides, the significant higher rates in TS-amended soil indicate the presence of an important amount of rapidly mineralizable compounds in the TS, that would be in lower proportion in the CS, since most of them have been already decomposed during the composting process (García et al., 1992).

III.3.2 Model fitting

Table III.2 shows the model parameters employed to examine the C-CO_2 emission data and the coefficient of determination (R^2) used to compare them. With the exception of the zero order equation for TS-amended soil, the kinetic models offer a good description of C mineralization kinetics for all the samples, especially the two simultaneous reactions and special models. Exponential and first-order E models present excellent descriptions of C mineralization kinetics in unamended and CS-amended soils but not so good for soil amended with TS.

The zero-order model shows the worst fit for the experimental data, probably because it doesn't consider the variation in the degradability of organic C with the incubation time. This result is in agreement with previous studies of other authors for organic wastes of different origin (Levi-Minzi et al, 1990; Ajwa and Tabatabai, 1994). C-CO_2 emission data for control and CS-amended soils fit well even to this model, which may be due to the very short initial phase of rapid degradation in this samples, characteristic of a well-mature organic matter. In contrast, TS-amended soil, with a more intense activity, shows better fits as the complexity of the models increases, i.e., as the ability of the model to accurately describe the variable degradation process increases. That's why the two simultaneous reactions model offers the best description for the three samples. This model describes mineralization curves considering two kinds of fractions of organic C with different degree of biodegradability: one fraction would be composed by labile organic compounds which are rapidly mineralized in soil while the other is mainly constituted by compounds resistant to microbial attack which broke down slowly during a second phase.

Values of rapidly mineralizable C (C_1) derived from the two simultaneous reaction model indicate a much larger presence of easily biodegradable organic compounds in TS-amended soil than in unamended and CS-amended soils. Rapidly mineralizable C in the last samples constitutes, respectively, the 6% and 11% of the total potentially mineralizable C (C_1+C_2), suggesting a great organic matter stability. Rate constants corresponding to this step (k_1) are exceptionally high for the control, which reflects the short time required to break down this small fraction. Values of k_1 obtained for CS- and TS-amended soils (0.873 d^{-1} and 0.296 d^{-1} respectively) are close to those calculated by Bernal et al. (1998) for a soil with mature compost of sewage sludge mixed with maize straw (0.795 d^{-1}) and for the same mixture analysed during the active phase of composting (0.295 d^{-1}).

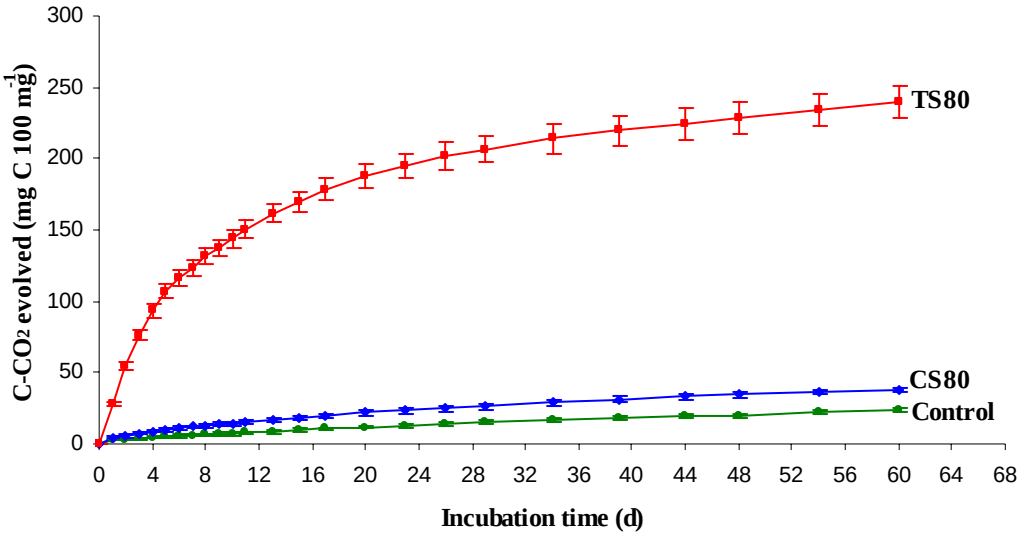

Figure III.2. Cumulative C released as CO_2 from unamended soil (Control) and soil amended with composted sewage sludge (CS80) or thermally-dried sewage sludge (TS80) during incubation. The bars indicate standard errors.

As C_2 values indicate, most of the organic C of the samples is slowly mineralized. This slow phase is particularly predominant in the case of the control and the CS-amended soil. According to the calculated rate constant (k_2), this step is relatively fast in comparison

Table III.2. Model parameters and coefficients of determination (R^2) for unamended soil (Control) and soils amended with either composted sewage sludge or thermally dried sewage sludge at a rate of 80 t ha^{-1} (CS80 and TS80, respectively).

Model	Parameter	**Treatment**		
(Reference)		**Control**	**CS80**	**TS80**
Zero-order	k	0.359	0.562	2.985
(Seyfried and Rao, 1988)	Intercept	3.558	8.144	98.486
	R^2	0.981	0.961	0.786
Exponential	k	1.769	4.267	61.481
(Levi-Minzi et al., 1990)	m	0.632	0.539	0.349
	R^2	0.998	0.999	0.958
First-Order	C_0	26.145	38.099	222.493
(Murwira et al., 1990)	k	0.032	0.046	0.109
	R^2	0.990	0.985	0.981
First-Order E	C_0	31.909	40.943	201.616
(Jones, 1984)	k	0.018	0.027	0.085
	C_1	2.071	4.451	27.600
	R^2	0.999	0.999	0.989
Two simultaneous reactions	C_1+C_2	34.369	47.625	246.360
(Molina et al., 1980)	C_1	2.134	5.315	94.594
	k_1	2.161	0.873	0.296
	C_2	32.235	42.310	151.766
	k_2	0.018	0.024	0.046
	R^2	0.999	0.999	0.999
Special	C_0	6.204	13.666	166.054
(Bonde and Roswall, 1987)	k_1	0.150	0.163	0.171
	k_2	0.295	0.426	1.298
	R^2	0.996	0.994	0.997

All the models have significant correlations coefficients at $P<0.001$

with those described by Bernal et al. (1998) for different mixtures of SS. However, values of k_1 for soil amended with TS are similar to those obtained for the C mineralization of soil amended with untreated SS (Pascual et al., 1998) and to those calculated by Ajwa and Tabatabai (1994) for a soil incubated with SS treated in an Imhoff tank, although in that case they applied a first-order model.

Table III.3 reports data relative to half-life time ($t_{1/2}$) and to the initial potential rate of C mineralization, (C_nk_n), a parameter that could be comparable with the initial potential rate of N mineralization indicated by Campbell et al. (1991). Several researchers have suggested that this product (C_nk_n) is more accurate to explain and understand the quality of SOM than either one separately (Murwira et al., 1990; Saviozzi et al., 1993). Values of initial potential rate of C mineralization for control and CS-amended soil suggest that both samples present organic compounds of similar decomposability, although during the second phase the quality of the organic matter added with CS, less humified than control SOM, favours the microbial attack, as is reflected by the higher C_2k_2 product and the shorter half-life time ($t_{1/2}$ = 28.5 d) of CS samples.

Table III.3: Parameters C_1k_1, C_2k_2 and half-life time, $t_{(1/2)1}$ and $t_{(1/2)2}$, estimated for unamended soil (Control) and soil amended with composted sewage sludge (CS80) or thermally-dried sewage sludge (TS80) at a rate of 80 t ha^{-1}.

	Control	CS80	TS80
C_1k_1 (mg C-CO_2 kg^{-1} sample d^{-1})	4.61	4.63	28.00
C_2k_2 (mg C-CO_2 kg^{-1} sample d^{-1})	0.58	1.03	7.00
$t_{(1/2)1}$ (d)	0.3	0.8	2.3
$t_{(1/2)2}$ (d)	38.5	28.5	15.0

Despite of the low values of k_1, the samples of soil amended with TS have the highest initial potential rate of C mineralization, which highlights the importance of the labile compounds and their intense degradation in the TS. Furthermore, the unusual high C_2k_2 product and the fairly short half-life time of this sample, in comparison with the huge quantity of C calculated for this pool, pointed to the existence of a still quite important microbial activity in this second phase. These results could be attributed to the presence of

intermediate products resulting of the maturation process that is taking place in the soil. In addition, due to the compact pellet form in which TS is applied, an important part of the materials which constitute the pellet, including the labile products, are being progressively accessible to microorganism at the same time that pellets break down, which also can slow down the mineralization process.

III.3.3 Mineralization parameters

Net mineralization coefficients are similar for control and CS-amended soil and significantly lower than the values obtained for soil amended with TS (**Table III.4**). After 60 days of incubation, CMC for soil amended with TS is significantly higher than for soil amended with CS. With respect to TS-amended soil, CS-amended soil shows lower values of DCMC with a constant and slight increase levelled off after 48 days of experiment (**Figure III.3**).

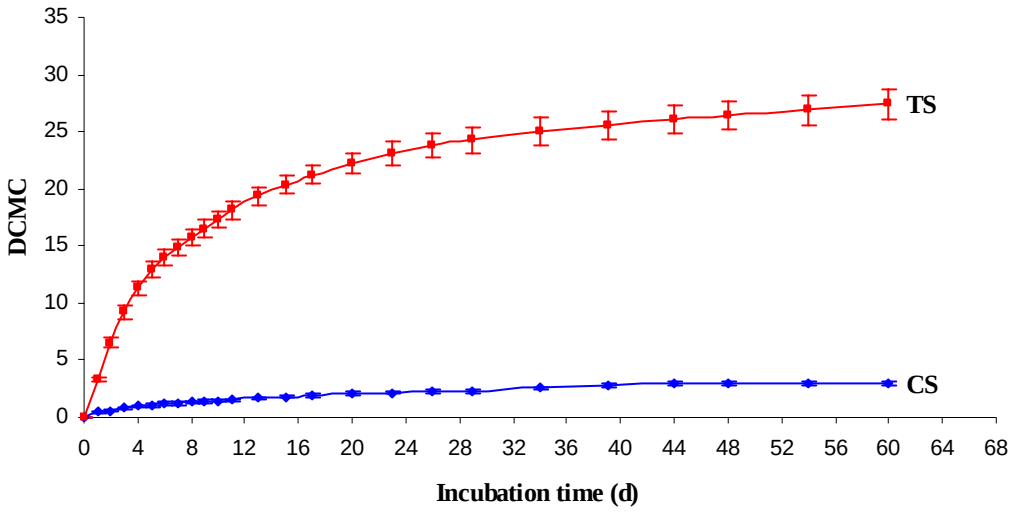

Figure III.3. Daily Complementary Mineralization Coefficient (DCMC) evolution of soil amended with composted sewage sludge (CS80) or thermally-dried sewage sludge (TS80) during incubation. The bars indicate standard errors.

Meanwhile soil amended with TS shows the highest growth rate during the first weeks, followed by a constant increase. The addition of CS makes a smaller contribution of organic matter than the input made by adding TS to the soil (**Table III.1**). However, NMC values obtained for CS-amended soil (**Table III.2**) show how the loss of C evolved as CO_2 is smaller than for TS-amended soil and similar to the control. This means that the organic matter contained in CS is very stable and similar to the SOM. Conversely, TSS-amended soil shows an important loss of organic C, possible due to the intense microbial degradation of the abundant labile organic fractions. According to the CMC results, more than 25% of the organic carbon from TS has been mineralized after 60 days, while this is less than 3% in the case of the CS. García et al. (1992) suggest that composts with a CMC of about 2.5% can be considered to possess a sufficiently stabilized organic matter. Hsieh et al. (1981) evaluated an activated sludge in soil where about 26% of organic C was mineralized.

Table III.4: Net Mineralization Coefficient (NMC) and Complementary Mineralization Coefficient (CMC) of Control and soil amended with composted sewage sludge (CS80) or thermally-dried sewage sludge (TS80) at a rate of 80 t ha^{-1}.

	Control	**CS80**	**TS80**
NMC (mg C-CO_2 100 mg^{-1} C sample)	2.79±0.08a	2.89±0.05a	14.83±0.40b
CMC (mg C-CO_2 100 mg $^{-1}$ C sludge)	-	2.95±0.24a	27.40±1.24b

Values of the same row followed by the same letter are not significantly different according to LSD test (P=0.05).

The analysis of the DCMC could be helpful to know when the new equilibrium in the soil microbial population is reached and when sowing may take place. Values of DCMC found for CS increase very slightly as incubation proceeds with a daily mineralization rate very similar to the control, suggesting that this amendment is mature enough for field application. However values of DCMC for TS still continue to increase after 60 days of incubation. These results reveal an extended microbial degradation of organic matter in TS-amended soil with respect to the basal levels of microbial activity in the unamended and CS-amended soil.

III.4. CONCLUSIONS

Soil amended with TS shows a greater degree of carbon mineralization because the organic matter of the TS is not stabilized yet, and the maturation process takes place in the soil. Meanwhile the C-CO_2 emission of the CS-amended soil is much less because CS has been submitted previously to a composting process. Due to its low mineralization this organic matter could give rise to a greater quantity of humus (García et al., 1992, Saviozzi, 1999, Fernández et al., 2007a) that will play important roles in soil (Stevenson, 1994). Meanwhile, nutrient supply is most closely associated with the active fractions of SOM, consequently the active pool of the TS is expected to provide more nutrients for soil microbes (Cooperband, 2002). Therefore, the use of TS as a fertilizer would promote the activation of the soil microorganism activity (Nannipieri et al., 1990, García et al., 1994b). This aspect is often negative for plant growth, since it may increase the mineralization rate of native soil organic C through extended microbial oxidation, and induce of anaerobic conditions by mineralization of large amounts of non-stabilized organic C with associated extended O_2-consumption, (Senesi and Brunetti, 1996; Plaza et al., 2003). In conclusion, if the goal is to restore SOM quickly, the use of TS as an organic amendment could result inefficient and the choice of a well-decomposed organic matter, like the kind present in the CS, should be recommended.

IV. ORGANIC MATTER IN DEGRADED AGRICULTURAL SOILS AMENDED WITH COMPOSTED AND THERMALLY-DRIED SEWAGE SLUDGES

IV. ORGANIC MATTER IN DEGRADED AGRICULTURAL SOILS AMENDED WITH COMPOSTED AND THERMALLY-DRIED SEWAGE SLUDGES

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INDEX

ABSTRACT

The cumulative and residual effects of composted and thermally-dried sewage sludge (CS and TS, respectively) on soil organic matter and its humified fraction were evaluated in a field experiment under Mediterranean conditions. The experimental design included soil plots either unamended (control) or amended with mineral fertilizer, CS and TS at rates of 20 and 80 t ha^{-1}. After the first year of sewage sludge application, each plot was divided into two subplots. In one subplot group, no additional application of SS was made in the following 3 years, and the residual effect of the first applications was evaluated. In the second subplot group, the cumulative effect of sludge amendments was evaluated by applying CS and TS also in the following three consecutive years. Nine months after the yearly sludge and mineral fertilizer applications, surface soil samples from control and amended soils were collected and analyzed for total organic C (TOC), total extractable C (TEC), and humified C fractions, both humic acid C (HAC) and fulvic acid C (FAC) fractions. Compared with the control and mineral treatments, which showed similar results, the repeated application to soil of TS, and specially CS, induced an increase on the content of the organic fractions examined, as well as HA percentage (%HA=HAC/TOC) and degree of polymerisation (DP=HAC/FAC). In the residual experiment, the TOC, TEC, HAC and FAC content of soils amended once with CS and TS decreased slightly when increasing the time from the amendment, whereas the %HA and DP tended to increase. Further, three years after the sludge applications, with respect to the control soil, the soils amended once with CS exhibited similar TOC, TEC, and FAC content, and slightly larger HAC content, %HA and DP; whereas those amended once with TSS, featured still larger TOC, TEC, HAC content, similar FAC content, and slightly larger %HA and DP values. As a whole, the results obtained suggested that both kind of sludges contribute to improved soil organic matter levels and humified fractions, although the CS contribution can be considered as a more efficient organic amendment than the TS, which may present problems of maturity and degradability.

Keywords: Sewage sludge, compost, thermally-dried, organic matter, humic substances.

IV.1. INTRODUCTION

Climatic conditions combined with inappropriate cultivation practices have produced a constant decrease of soil organic matter (SOM) contents in several semiarid Spanish Mediterranean areas, which constitutes one of the most important causes of soil degradation in these agroecosystems (García et al., 1994a; García-Gil et al., 2000). It is well known that the main contribution of SOM to soil fertility and plant growth is indirect by improving a wide number of soil physical, chemical and biological properties (Stevenson, 1994). Most of these beneficial aspects can be attributed to humic substances, and especially to the humic acids (HAs), which represent the most abundant and chemically significant fraction of SOM (Senesi, 1989). Along with their importance in nutrient exchange, HAs are known to promote a good soil structure, thus improving aeration, moisture retention and soil protection from degradation and erosion (Stevenson, 1994). For these reasons, agricultural practices based in periodical inputs of stable, humified organic amendments are strongly recommended to increase and restore SOM, particularly in the fragile Mediterranean agroecosystems.

Treatment of municipal wastewater leads to the generation of huge volumes of sewage sludge (SS), whose disposal represents one of the most environmentally challenging issues of the wastewater treatment process (Albiach, 2001). As other several organic residues produced by anthropic activities, SS is a potential candidate to be recycled as a soil amendment, which is considered the most environmentally-safe, economically-advantageous disposal option, based on the agronomic benefit of adding nutrients and organic matter to soil (Haug, 1993; Pascual et al., 1999). However, before being applied in agriculture, raw SS should be subjected to appropriate treatments designed to enhance the stability of the organic matter, in order to improve its potential as a organic fertilizer and, at the same time, avoid a number of harmful effects on soil properties and health hazards that may result from its use (Senesi and Brunetti, 1996; Plaza et al., 2003).

Typically, raw SS is firstly dewatered by centrifuges and presses to produce an easy-to-process and transport sludge cake. Among the various further treatments to produce an

environmentally-safe and agronomically-advantageous soil organic amendment at an acceptable operational cost is the composting process. Composting consists of a controlled biological decomposition of organic matter under aerobic conditions, and the transformation of the residual organic matter into stabilized, refractory organic materials, the so-called "humic-like" substances that chemically resemble native soil humic substances (Senesi, 1989; Francis, 1998). A relatively newer process is thermal drying that uses intense heat, thereby inactivating pathogens, removing volatile chemicals and leading to a final product in pellets with low odour and good handling characteristics.

To ensure the sustainability of soil and the safe and efficient use of composted and thermally-dried SS, as well as to monitor and improve the performance of SS management systems, it is necessary to control and research the type and extent of SOM changes induced by the application of these amendments. However, the content of organic matter in itself should not be used alone, other more sensitive indicators such as the HA- and FA-C contents are needed (Sparkling, 1992). Furthermore, several indices that relate these parameters have shown to be valuable tools for evaluating SOM changes (Iglesias et al., 1989; Senesi, 1989).

The objective of this work was to investigate, using chemical parameters, the SOM changes and evolution in a degraded Mediterranean soil amended with composted sewage sludge (CS) or with thermally-dried sewage sludge (TS).

IV.2. MATERIALS AND METHODS

The CS used in this experiment was collected from a compost pile containing a mixture of SS from three different municipal wastewater treatment plants in the Madrid (Spain) metropolitan area and that have been subjected to a conventional composting process. The TS sample was collected from the wastewater treatment plant SUR of Madrid metropolitan area where SS is dried in a process that uses hot air at high temperatures (between 380ºC and 450ºC) with a final product in pellets of about 2.5 mm of diameter. The field experiment was conducted in the CSIC experimental farm "La Higueruela" located in Toledo (Spain) on a sandy loam soil (sand, 580 g kg^{-1}; silt, 240 g kg^{-1}; clay, 180 g kg^{-1})

classified as a Typic Haploxeralf (Soil Survey Staff, 2003). The site is characterized by a continental semiarid climate with an average annual rainfall of about 487 mm and an average annual temperature of 14ºC. The main chemical properties of the CS, TS and soil are included in **Table IV.1**.

Table IV.1: Main chemical properties of soil, composted sewage sludge and thermally-dried sewage sludge used in the experiment.

	Soil	Composted Sewage Sludge	Thermally-dried Sewage Sludge
Dry matter (g kg^{-1})		435.8	846.4
pH (H_2O)	5.7	7.1	7.0
Electrical Conductivity (dS m^{-1})	0.05	3.90	1.50
Total Organic Carbon (g kg^{-1})	7.2	181	296
Total Extractable Carbon (g kg^{-1})	1.4	53.4	87.7
Humic Acids Carbon (g kg^{-1})	0.7	21.0	24.8
Fulvic Acids Carbon (g kg^{-1})	0.6	32.4	62.9
C/N	8.0	7.6	8.3
Total nitrogen (g kg^{-1})	0.9	23.9	35.6
Total phosphorus (g kg $^{-1}$)	0.09	13.90	13.43
Total potassium (g kg^{-1})	0.20	5.02	4.29

The experimental design was performed for three years (2002-2004) in four randomised blocks of nonirrigated soil plots (20 x 3 m^2) cropped with barley (*Hordeum vulgare* L.). Both SS were applied in mid-September, prior to barley planting in mid-October, and immediately incorporated into soil at a depth of 0-15 cm at two rates: 20 dry matter t ha^{-1} (CS20 and TS20, respectively) and 80 dry matter t ha^{-1} (CS80 and TS80, respectively). An unamended control soil and a soil fertilized with 400 kg ha^{-1} of mineral fertilizer (15-15-15) were used to compare with the amended soils. After first year of sludge treatment each plot was divided in half. In one part, the same amount of sludge was applied yearly until 2004 in order to study the cumulative effects of the amendments. In the other part, no addition was made in the following two years with the aim of evaluating the residual effects of the first application. Soon after barley harvest (late June), surface soil subsamples were collected randomly from the arable layer (Ap horizon, 0-20 cm depth) from each plot.

Each soil subsample consisted of a mixture of 10 soil cores each of 3-cm diameter. A composite sample was then obtained for each treatment by mixing equal amounts (1 kg) of the four corresponding soil subsamples.

Prior to analysis samples were air-dried and passed through a 2 mm sieve. The principal chemical properties of soils and sludges were determined by the Official Method of Analysis of the Spanish Ministry of Agriculture (MAPA, 1994). In particular, the pH was measured on mixtures of soil:water = 1:2.5 and sludge:water = 1:5; the electrical conductivity was measured on a 1:5 sample:water extract; and the total organic carbon (TOC) was determined by dichromate oxidation of the sample and subsequent titration with ferrous ammonium sulphate. Further, total extractable carbon (TEC) content was obtained by extraction from the sample with 0.1 M $Na_4P_2O_7$ (pH 9.8), using a sample:extractant ratio of 1:10 and subsequent colorimetric determination (Sims and Haby, 1971). An aliquot of the extract was acidified with HCl to pH 1, left standing for 24 h in a refrigerator to allow the complete precipitation of HA, and then centrifuged. Fulvic acids content (FAC) was determined by measuring of C content in the supernatant in the same way explained above for TEC. Humic acid content (HAC) was calculated by subtracting the C content in the acidified supernatant from the TEC content. The humification ratio (HR), percentage of humic acids (%HA) and degree of polymerisation (DP) were calculated from these parameters as (TEC/TOC) x 100, (HAC/TOC) x 100 and HAC/FAC, respectively (Senesi, 1989).

Analytical data were subjected to a one-way ANOVA test. Mean separation was performed with the least significant difference (LSD) test when F-test was significant at a 0.05 probability level. Statgraphics Plus 2.0 software for Windows was used for statistical analysis (Statistical Graph Corp. 1996).

IV.3. RESULTS AND DISCUSSION

IV.3.1. Soil organic matter contents

Figure IV.1 shows the effect of the various treatments on the contents of TOC and TEC in soil and **Figure IV.2** on the contents of HAC and FAC. There are not significant differences between the control soil and the mineral fertilized soil in the contents of the different fractions of SOM examined. However, with respect to the control soil, the applications of both CS and TS for three years markedly increased the contents of TOC, TEC, HAC, and FAC. These effects are generally more evident with increasing the amendment rate and number, and are slightly more evident for the CS-amended soils than for the TSS-amended ones.

Despite the larger TOC, TEC, HAC, and especially FAC contents of TS, as compared to CS, the repeated application to soil of TSS for 1, 2, and 3 years appears to increase in a slightly less extent the contents of the different fractions of SOM examined, which suggests the occurrence of more extended mineralisation processes in these soils than in the CS-amended ones. This behaviour may be attributed to the larger presence of easily biodegradable compounds in TS than in CS. Unlike the CS, TS is not subjected to a maturation process, only to a standstill of the microbial activity due to high temperatures, which might be reactivated when TS is applied to soil due to favourable conditions of moisture and temperature.

In contrast to the trends observed in the cumulative experiment, the contents of TOC, TEC, HAC, FAC of soils subjected to residual treatments tend to decrease slightly with increasing the time from the amendment, especially the CS-amended soils. The decrease is more marked for the FAC fraction than for the other ones, which may be attributed to its higher degradability, and/or to its transformation to more complex molecules such as HAs (Boyd et al., 1980; De Nobili et al., 1988). Further, after three years from sludge applications, the soils amended once with TS exhibit similar or slightly larger TOC, TEC, HAC, and FAC contents than those amended once with CS and the unamended control soil. This apparent greater residual effect of TS as compared to CS after three years from the amendments may be attributed to the physical resistance of the pellets to be decomposed, which means that a great part of the material are not available to be degraded or incorporated to the soil.

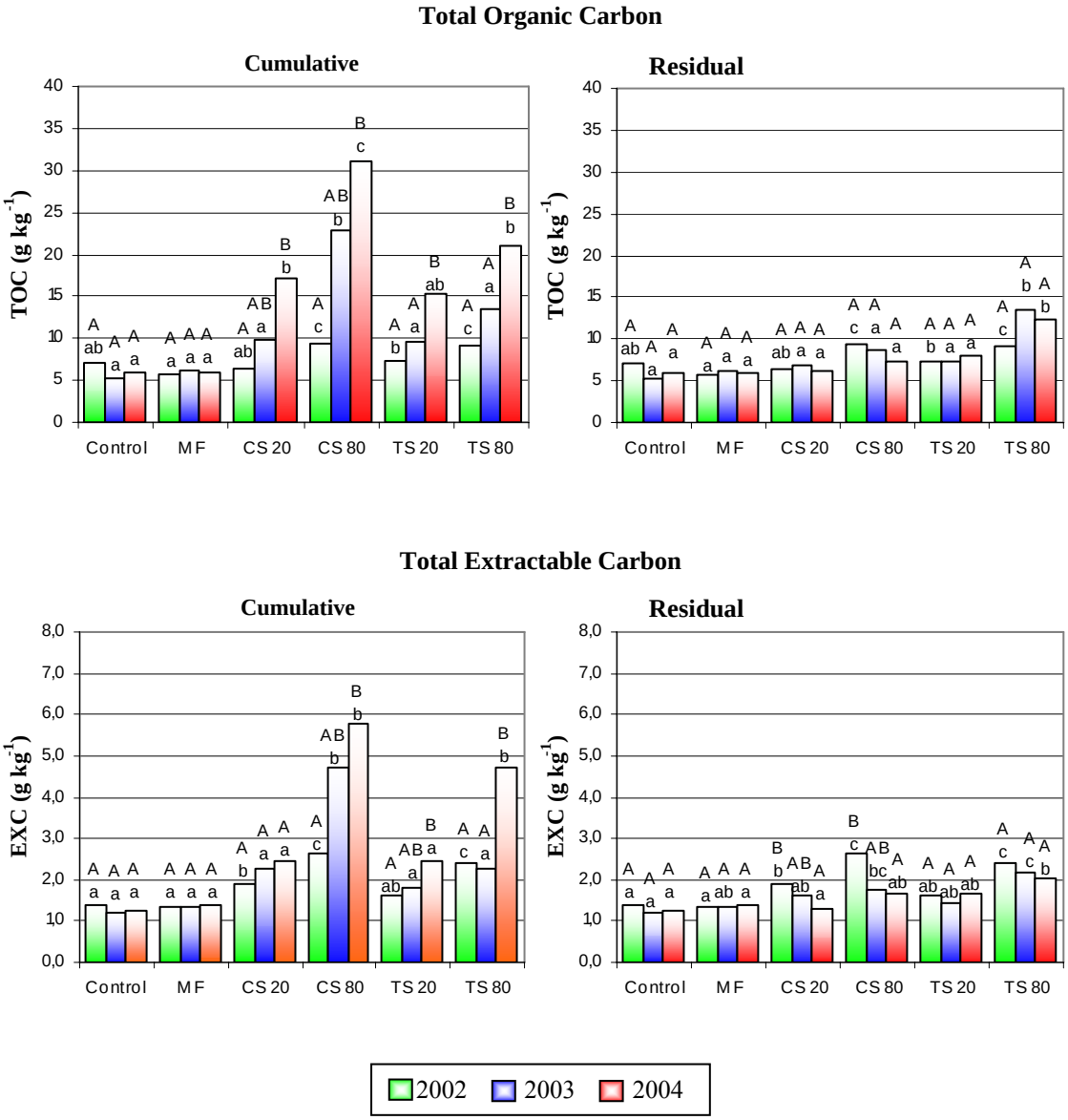

Figure IV.1: Total organic carbon (TOC) and total extractable carbon (TEC) contents of control and soils amended with mineral fertilizer (MF), composted sewage sludge at rates of 20 t ha^{-1} or 80 t ha^{-1} (CS20 and CS80, respectively) and thermally-dried sewage sludge at rates of 20 t ha^{-1} or 80 t ha^{-1} (TS20 and TS80, respectively) in cumulative and residual treatments. Bars of same year with the same lose case letter are not significantly different according to LSD test (P = 0.05). Bars of same treatment with the same capital letter are not significantly different according to LSD test (P = 0.05).

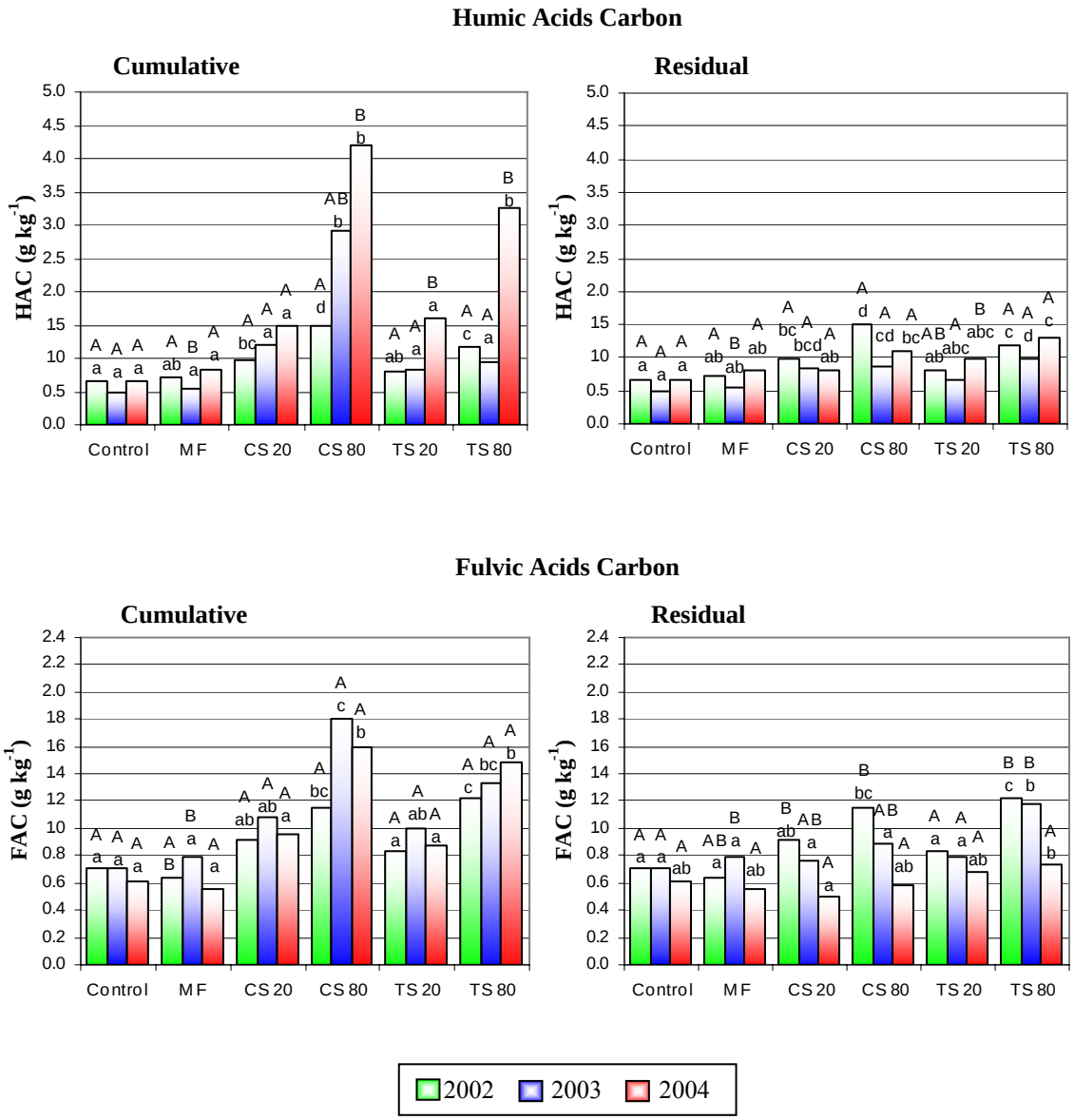

Figure IV.2: Humic acids carbon (HAC) and fulvic acids carbon (FAC) contents of control and soils amended with mineral fertilizer (MF), composted sewage sludge at rates of 20 t ha^{-1} or 80 t ha^{-1} (CS20 and CS80, respectively) and thermally-dried sewage sludge at rates of 20 t ha^{-1} or 80 t ha^{-1} (TS20 and TS80, respectively) in cumulative and residual treatments. Bars of same year with the same lose case letter are not significantly different according to LSD test (P = 0.05). Bars of same treatment with the same capital letter are not significantly different according to LSD test (P = 0.05).

IV.3.2. Humification parameters

The humification parameters (HR, %HA, DP) of unamended soil, mineral fertilized soil and soils amended with either CS or TS at rates of 20 or 80 t ha^{-1} in the cumulative and residual treatments are shown in **Tables IV.2** and **IV.3**, respectively. Compared with the control and mineral treatments, which generate almost similar results, the repeated application to soil of CS and TS induce a decrease of the %HA and DP, and an increase of the HR. Except for the HR, for which the contrary is true, these effects tend to increase with increasing the amount of sludge applied to soil per year and the number of years of sludge application, and appear to be less pronounced for the TSS-amended soils than for the CSS amended ones.

In the residual experiment, the HR of soils amended once with CS and TS decrease slightly with increasing the time from the amendment, whereas the %HA and DP tend to increase. These latter results are consistent with the trend for FAC discussed above in suggesting the formation of complex molecules (HAs) from more simple molecules (FAs). After three years from sludge applications, with respect to the unamended soil, the sludge-amended soils feature similar HR, but slightly larger %HA and DP.

With the exception of HR, the humification indexes appears to indicate that the application of both CS and TS increase similarly the humification degree of SOM in the amended soils, which represents a positive result in the context of beneficial recycling of SS. The opposite conclusion suggested by the HR may be attributed to an overestimation of the TEC content. It should be noted that alkaline extractants to isolate and solubilize the humic substances could also dissolved protoplasmic and structural components of plant residues and microbial biomass. So when humic substances are extracted from organic wastes, others substances with few humic characteristics are extracted too (Iakimenko et al., 1996). These include lignin residues, quinones, polyphenols, fats, dibenzocarboxylic acids, as well as aliphatic-type residues such as carboxylic and fatty acids, alkanes, polysaccharides, simple peptides, etc. (Sánchez-Monedero et al., 1999).

Table IV.2: Humification ratio, % humic acids and degree of polymerisation of control and soils amended with mineral fertilizer (MF), composted sewage sludge at rates of 20 t ha^{-1} or 80 t ha^{-1} (CS20 and CS80 respectively) and thermally-dried sewage sludge at rates of 20 t ha^{-1} or 80 t ha^{-1} (TS20 and TS80 respectively) in cumulative treatments.

	Humification Ratio			% Humic acids			Degree of Polymerisation		
	2002	2003	2004	2002	2003	2004	2002	2003	2004
Control	19.36a A	25.21c B	21.60ab BC	47.38a B	39.89a A	51.85a B	0.91a B	0.67a A	1.08a B
MF	23.89abc A	22.56bc A	23.50b A	52.63a B	41.36ab A	60.13bc C	1.11ab B	0.71a A	1.51ab C
CS20	29.60d B	23.60bc A	21.84ab A	52.57a A	52.10d A	62.40bc B	1.15ab A	1.09d A	1.67bcd B
CS80	28.12cd A	21.67abc A	23.92b A	55.85a AB	48.08cd A	64.47c B	1.33b AB	0.93cd A	1.85d B
TS20	22.17ab A	19.54ab A	23.31b A	49.00a A	45.09bc A	59.22b B	0.98ab A	0.83abc A	1.46b B
TS80	26.58bcd B	16.40a A	16.94a A	49.23a A	45.73bc A	64.08c B	0.98ab A	0.84bc A	1.79cd B

Values of the same column followed by the same lose case letter are not significantly different according to LSD test ($P = 0.05$).

Values of the same row followed by the same capital letter are not significantly different according to LSD test ($P = 0.05$).

Table IV.3: Humification ratio, % humic acids and degree of polymerisation of control and soils amended with mineral fertilizer (MF), composted sewage sludge at rates of 20 t ha^{-1} or 80 t ha^{-1} (CS20 and CS80 respectively) and thermally-dried sewage sludge at rates of 20 t ha^{-1} or 80 t ha^{-1} (TS20 and TS80 respectively) in residual treatments.

	Humification Ratio			% Humic acids			Degree of Polymerisation		
	2002	2003	2004	2002	2003	2004	2002	2003	2004
Control	19.36a A	25.21b B	21.60bc BC	47.38a B	39.89a A	51.85a B	0.91a B	0.67a A	1.08a B
MF	23.89abc A	22.56ab A	23.50c A	52.63a B	41.36a A	60.13b C	1.11ab B	0.71a A	1.51ab C
CS20	29.60d B	24.86b B	15.36a A	52.57a A	52.16b A	60.90b B	1.15ab A	1.09b A	1.57ab B
CS80	28.12cd B	21.44ab B	18.27ab A	55.85a A	60.56c A	70.65d B	1.33b A	1.56c A	2.53cd B
TS20	22.17ab C	19.11a B	16.24a A	49.00a A	44.32a A	64.27bc B	0.98ab A	0.80a A	1.82bc B
TS80	26.58bcd A	19.97ab A	23.59c A	49.23a B	41.35a A	68.67cd C	0.98ab A	0.72a A	2.20d B

Values of the same column followed by the same lose case letter are not significantly different according to LSD test ($P = 0.05$).

Values of the same row followed by the same capital letter are not significantly different according to LSD test ($P = 0.05$).

IV.4. CONCLUSIONS

In general, the amendment with SS leads to an increase of SOM content and humic fractions, as well as an improvement of the humification parameters, which is expected to have an important and positive impact on soil quality and fertility. Despite the quantity of C applied to soil was much larger in the case of the TS, it has contributed less than CS to increase the different SOM fractions examined in SS-amended soils, suggesting a higher mineralization process in TS-amended soil

V. LONG-TERM EFFECTS OF AMENDMENT WITH COMPOSTED AND THERMALLY-DRIED SEWAGE SLUDGES ON SOIL AND HUMIC ACID PROPERTIES

V. LONG-TERM EFFECTS OF AMENDMENT WITH COMPOSTED AND THERMALLY-DRIED SEWAGE SLUDGES ON SOIL AND HUMIC ACID PROPERTIES

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INDEX

ABSTRACT

The effect of annual additions of composted sewage sludge (CS) or thermally-dried sewage sludge (TS) at a rate of 80 t ha-1 on soil chemical properties was investigated during three years in a field experiment under semiarid conditions. Further, humic acids (HAs) isolated by conventional procedures from CS and TS and unamended (SO) and sludge amended soils were analysed for elemental (C, H, N, S and O) and acidic functional group (carboxylic and phenolic) composition, and by ultraviolet/visible, Fourier transform infrared and fluorescence spectroscopies. With respect to CS, TS had similar pH and total P and K contents, larger dry matter, total organic C and total N contents and C/N ratio, and smaller ash content and electrical conductivity. Amendment with either sludge induced a number of modifications in soil properties, including an increase of pH, electrical conductivity, total organic C, total N, and available P. The CS-HA had greater O, total acidity, carboxyl and phenolic OH group contents and smaller C and H contents than TS-HA. The CS-HA and TS-HA had larger N and S contents, smaller C, O and acidic functional group contents, and lower aromatic polycondensation and humification degrees than SO-HA. Amended soil-HAs showed C, H, N and S contents larger than SO-HA which suggested that sludge HAs were partially incorporated into soil HAs. These effects were more evident with increasing number of sludge applications.

Keywords: Sewage sludges; Soil amendment; Humic acids; Elemental composition; Functional groups; Fourier transform infrared spectroscopy; Fluorescence spectroscopy.

V.1. INTRODUCTION

The typically low natural levels of soil organic matter in Mediterranean areas are often lowered by inappropriate agricultural practices, which affect negatively crop yields and increase the risk of erosion and desertification in these areas (Singer and Le Bissonnais, 1998; Caravaca et al., 2002; Plaza et al., 2004; Senesi et al., 2006). In general, soils poor in organic matter benefit of organic amendment that can contribute to both maintaining the global soil fertility status and related agricultural production level and protecting the soil from degradation and contamination (Schnizter, 1991; Stevenson, 1994; Senesi and Loffredo, 1999; Deng and Dixon, 2002). However, the limited availability of organic matter resources traditionally employed in agriculture, such as manure, cannot satisfy the current soil requirements, and new sources, including organic wastes of various nature, are being experimented. Among these, urban sewage sludges (SS), which are rich in organic matter, N and P, are the most abundant and frequently used. Further, SS recycling in agriculture represents also a convenient alternative to their landfill disposal.

In general, raw SS must be submitted to appropriate treatments before application to soil, in order to enhance its agronomic efficiency and avoid possible harmful effects on soil properties and health (Senesi et al., 1996; Plaza et al., 2003). These effects, however, may vary widely depending especially on the SS nature and the treatment to which SS is subjected before its application to soil. Composting is one of the most common and traditional SS treatment, and consists in the biological oxidation of raw SS by indigenous microorganisms that leads to a chemically stabilized, biologically mature and sanitized final product, free of pathogens and plant seeds, rich in humic-like substances, easy to be stored, and marketable as organic amendment or fertilizer (Stevenson and He, 1990; Haug, 1993; Ouatmane et al., 2002). Thermal drying is another relatively recent and increasingly used SS treatment that consists in subjecting SS to a short and intense heating able to destroy pathogens, eliminate volatile chemicals and lead to a sanitized, pelleted final product with good handling characteristics but lacking of a maturation process.

The control of the chemical quality of organic matter, and especially of the HA-like fraction in treated SS is of crucial importance to ensure an environmentally safe and agronomically efficient use of SS as soil amendment. In particular, before application to soil, the HA-like fraction in treated SS should resemble as much as possible native soil HA (Senesi et al., 1996). Further, the knowledge of the type and extent of changes caused in soil HA by SS application is also essential for a correct evaluation of the SS value as an organic amendment.

The main objectives of this work were: (a) to determine and discuss comparatively the molecular, structural and functional properties of the HA-like fractions isolated from CS and TS sludges; and (b) to investigate the effect of their cumulative applications on the properties of soil HAs. To reach these objectives chemical methods combined with spectroscopic techniques, including elemental and functional group analyses and ultraviolet/visible (UV-Vis), Fourier transform infrared (FTIR) and fluorescence spectroscopies, were used.

V.2. MATERIALS AND METHODS

V.2.1. Sewage sludges, soil and field experiment

The CS sample was collected from a 3-month windrow-composted mixture of three SS originated from three municipal wastewater treatment plants in Madrid (Spain) metropolitan area. The TS sample consisted of a SS dried with hot air at high temperatures (between 380ºC and 450ºC) in the wastewater treatment plant SUR in Madrid metropolitan area.

The field experiment was conducted in the experimental farm “La Higueruela” located in Toledo (Spain) on a sandy loam soil (sand, 580 g kg^{-1}; silt, 240 g kg^{-1}; clay, 180 g kg^{-1}) classified as a Typic Haploxeralf (Soil Survey Staff, 2003). The site is characterized by a continental semiarid climate with an average annual rainfall of about 487 mm and an average annual temperature of 14 ºC.

The experimental design consisted in four random blocks of nonirrigated soil plots (20 x 3 m^2) cropped with barley (*Hordeum vulgare* L.), either unamended (SO) or amended yearly for 3 years (from 2002 to 2004) at a rate of 80 t ha^{-1} with CS (CS80-02, CS80-03 and CS80-04, respectively) or TS (TS80-02, TS80-03 and TS80-04, respectively). Both SS were applied in mid-September, prior to barley planting in mid-October, and immediately incorporated into soil at a depth of 0-15 cm. Soon after barley harvesting in late June, surface soil subsamples (Ap horizon, 0-15 cm depth) were collected randomly from each plot. Each soil subsample consisted of a mixture of 20 soil cores each of 3-cm diameter. A composite sample was then obtained for each treatment by mixing equal amounts (1 kg) of the four corresponding soil subsamples.

V.2.2. Sewage sludge and soil analyses

Prior to analyses, soil and SS samples were air-dried and passed through a 2 mm sieve after removal of plant residues and stones from soils. The principal chemical properties were determined according to standard methods (Sparks et al., 1996) in triplicate analysis of each sample.

In particular, dry matter and ash contents of SS were measured after heating the samples overnight at 105 ºC or 550 ºC, respectively. The pH was measured on mixtures of soil:water = 1:2.5, and SS:water=1:5. Electrical conductivity (EC) was measured on a 1:5 sample:water mixture. Total organic carbon (TOC) was determined by dichromate oxidation of the sample and subsequent titration with ferrous ammonium sulphate (Walkley and Black, 1934). Total N content was obtained by mineralization of the sample by the Kjeldahl method and subsequent colorimetric determination (Hinds and Lowe, 1980) by a Technicon Autoanalyzer AAII (Buffalo Grove, Il, USA). The P and K contents were measured by inductively coupled plasma-atomic emission spectrometry on a nitric and perchloric acid digest of each sample. The main chemical properties of CS and TS and unamended and SS-amended soils are presented in **Table V.1** and **Table V.2**, respectively.

Table V.1. Main chemical properties (± standard error) of the composted sewage sludge (CS) and the thermally-dried sewage sludge (TS).

Property	CS	TS
Dry Matter (g kg^{-1})	435.8±0.9	846.4±0.9
Ash (g kg^{-1})[a]	616.4±0.1	435.3±0.1
pH	7.1±0.1	7.0±0.1
Electrical Conductivity (dS m^{-1})	3.90±0.01	1.50±0.02
Total Organic C (g kg^{-1})	181.0±0.2	296.0±0.2
Total N (g kg^{-1})	23.9±0.1	35.6±0.1
Total P (g kg^{-1})	13.90±0.02	13.43±0.02
Total K (g kg^{-1})	5.02±0.07	4.29±0.02
C/N	7.6±0.2	8.3±0.2

[a] On dry-matter basis.

V.2.3. Humic acid isolation

The HA fractions were isolated from unamended and SS-amended soils and CS and TS samples by conventional methods. Prior to extraction of HA, carbonates were removed from soil samples by mechanical stirring with 2M H_3PO_4 for 30 min. The treatment was repeated three times, and then the samples were washed with distilled water until the suspension reached a pH of 7.

Briefly, free-carbonate soils and SS samples were first extracted with 0.1 M $Na_4P_2O_7$ at pH 9.8 and then by 0.1 M NaOH, at room temperature (RT, about 293 K), and using a sample to extractant ratio of 1:10. Each extraction was repeated three times by shaking mechanically the mixture for 3 h, then centrifuging at 15300 x g for 15 min, and filtering the supernatant through a Whatman no. 31 filter paper. The combined alkaline supernantants were then acidified with HCl until reaching a pH=1, left standing for 24 h in a refrigerator to allow the complete precipitation of HA, centrifuged at 30100 x g for 15 min, and then filtered through a Whatman no. 31 filter paper. The HA precipitates were then purified by dissolution in 0.1 M NaOH, centrifugation at 30100 g, elimination of the residue, acidification of the alkaline supernatant with HCl until pH 1, and standing for 12 h in a

refrigerator. The purification procedure was repeated three times. The precipitated HAs were then recovered with distilled water, dialyzed until free of Cl^- ions, and finally freeze-dried.

Table V.2. Some chemical properties (± standard error) of unamended soil (SO) and soils amended with composted sewage sludge (CS) or thermally-dried sewage sludge (TS) at a rate of 80 t ha^{-1} y^{-1} for 1, 2 and 3 years (CS80-02, CS80-03, CS80-04 and TS80-02, TS80-03 and TS80-04, respectively).

Sample	pH	EC	TOC	Total N	Available P	Available K	C/N
		(dS m^{-1})	(g kg^{-1})				
SO	5.7±0.1	0.05±0.01	7.2±0.1	0.9±0.1	0.09±0.01	0.20±0.01	8.0±0.1
CS80-02	6.4±0.2	0.10±0.01	9.5±0.1	1.0±0.1	0.03±0.01	0.17±0.02	8.8±0.2
CS80-03	6.4±0.2	0.27±0.02	22.8±0.5	2.8±0.3	0.21±0.02	0.21±0.03	8.0±0.1
CS80-04	6.5±0.1	0.29±0.01	31.0±0.5	4.5±0.3	0.58±0.02	0.31±0.03	7.0±0.5
TS80-02	5.8±0.1	0.18±0.03	9.1±0.3	1.4±0.1	0.03±0.01	0.17±0.01	6.8±0.2
TS80-03	5.8±0.1	0.14±0.04	12.3±0.5	1.7±0.2	0.14±0.02	0.18±0.01	7.5±0.4
TS80-04	6.2±0.1	0.12±0.02	20.3±0.5	3.0±0.4	0.16±0.03	0.25±0.02	7.5±0.5

EC: Electrical conductivity; TOC: Total organic carbon.

V.2.4. Humic acid analyses

Moisture content of HAs was measured by heating overnight at 105 ºC and ash content by burning overnight at 550 ºC. The C, H, N and S contents were determined in triplicate using a Fisons Instruments (Crawley, UK) elemental analyser model EA 1108. Oxygen content was calculated by difference: O%= 100 – (C+H+N+S) %. Carboxyl group content was estimated by direct potentiometric titration as the value of charge at pH 8, phenolic group content was estimated as two times the change in charge between pH 8 and pH 10, and total acidity was calculated by addition (Ritchie and Perdue, 2003).

Absorbances at 465 and 665 nm were measured on solutions of 3.0 mg of each HA in 10 ml of 0.05 M $NaHCO_3$, with pH adjusted to 8.3 with 0.02 M NaOH, using a Perkin Elmer (PE; Norwalk, CT) Lambda 15 UV/Vis spectrophotometer (Chen et al., 1977). The ratio of absorbances at 465 and 665 nm gave the E_4/E_6 ratio.

The FT IR spectra of HAs were recorded over the range from 4000 to 400 cm^{-1} on pellets obtained by pressing under reduced pressure a mixture of 1 mg of HA and 400 mg of dried KBr, spectrometry grade. A Nicolet (Madison, WI) 5PC FT IR spectrophotometer operating with a peak resolution of 2 cm^{-1} and Omnic 1.2 software were used to obtain spectra.

Conventional monodimensional fluorescence spectra in the emission, excitation and synchronous-scan modes and three-dimensional fluorescence spectra in the form of excitation-emission matrix (EEM) plots were obtained on aqueous solutions of HA at a concentration of 100 mg L^{-1} after overnight equilibration at RT, and adjustment to pH 8 with 0.05 M NaOH. All spectra were recorded using a Perkin Elmer (Norwalk, CT) LS-55 luminescence spectrophotometer equipped with the WinLab 4.00.02 software (PE, Inc. 2001, Norwalk, CT) for data processing. Emission and excitation slits were set at a 10 nm bandwidth, and a scan speed of 500 nm min^{-1} was selected for both monochromators. Emission spectra were recorded over the range 380-550 nm at a constant excitation wavelength of 360 nm. Excitation spectra were obtained over a scan range 300-500 nm by measuring the emission radiation at a fixed wavelength of 520 nm. Synchronous-scan excitation spectra were measured by scanning simultaneously both the excitation (varied from 300 to 550 nm) and the emission wavelengths, while maintaining a constant, optimised wavelength difference $\Delta\lambda = \lambda exc - \lambda em = 18$ nm (Senesi et al., 1991). To generate EEMs series of emission spectra were collected between 350 to 550 nm while the excitation wavelength increased sequentially by 5 nm from 300 to 550 nm. The EEM plots were generated from fluorescence spectral data using the Surfer 8.01 software (Golden Software, Inc., 2002, Golden, CO).

V.3. RESULTS AND DISCUSSION

V.3.1. Sewage sludges and soils

The chemical properties of CS (**Table V.1**) generally fall within the ranges commonly reported in the literature for this kind of materials (García et al., 1992; Saviozzi; 1999,

Albiach et al., 2001). With respect to CS, TS has much larger dry matter, TOC and total N contents, similar pH, C/N ratio and total P and K contents, and smaller ash and EC values.

The soil SO features a slightly acidic pH, small EC, and low contents of TOC, N, P and K (**Table V.2**). With respect to SO, the SS-amended soils show generally larger pH and EC values and TOC, total N and available P contents. These effects, which are more evident with increasing the number of SS additions, may be related directly to the high contents of organic matter, N, P and soluble salts in both SS applied.

The CS- and TS-amended soils show similar pH and available K, and variable C/N ratios. Despite the larger TOC, N and P contents of TS as compared to CS, TS-amended soils show values of these parameters smaller than those of CS-amended soils. These results thus suggest the presence of a larger amount of easily degradable organic compounds in TS than in CS, and the occurrence of more extended mineralization processes in TS-amended soils than in CS-amended soils (García-Gil et al., 2004; Fernández et al., 2007b).

V.3.2. Humic acids

V.3.2.1. Ash, elemental and functional group composition and E_4/E_6 ratio

The elemental and acidic functional group composition of HAs isolated from CS and TS differs between each other and from that of SO (**Table V.3** and **Table V.4**). In particular, CS-HA has larger O, carboxyl and, especially, phenolic OH group contents and smaller C and H contents and C/N ratio than TS-HA. These results suggest that, with respect to thermal drying, composting favours the formation of a HA fraction more oxidized and richest in acidic functional groups, typical of a more mature organic material (Senesi et al., 1996). Similar results for composted vs. uncomposted SS are reported by other authors (Boyd et al., 1980; Gerasimowicz and Byler, 1985).

With respect to SO-HA, CS-HA and TS-HA have smaller or much smaller C, O and COOH and phenolic OH group contents and C/N ratio, and larger or much larger N and S

contents. The H content of CS-HA is similar to that of SO-HA whereas that of TS-HA is much larger. The high levels of N and S in SS-HAs may be ascribed to the incorporation of protein decomposition products and S-containing surfactant residues in HA macromolecules. Further, the low content of acidic functional groups, particularly COOH groups, indicates a low degree of humification of SS-HAs as compared to SO-HA, and the low level of phenolic groups may be related to the generally lower amount of lignin structures present in SS (Senesi et al., 1996).

Table V.3 Elemental composition and atomic ratios (± standard error) of humic acids isolated from the unamended soil (SO), composted sewage sludge (CS), thermally-dried sewage sludge (TS) and soils amended with CS or TS at a rate of 80 t ha^{-1} y^{-1} for 1, 2 and 3 years (CS80-02, CS80-03, CS80-04 and TS80-02, TS80-03 and TS80-04, respectively).

Origin of HA sample	C	H	N	S	O	C/N	C/H	O/C
	($g\ kg^{-1}$)[a]					(Atomic ratios)		
SO	505±6	53±3	44±1	4±0	394±9	13.3	0.8	0.6
CS	460±1	53±1	85±0	14±0	389±1	6.3	0.7	0.6
CS80-02	526±6	61±2	52±1	9±0	352±7	11.8	0.7	0.5
CS80-03	519±3	59±0	85±0	11±2	326±5	7.1	0.7	0.5
CS80-04	598±0	67±0	90±0	16±0	228±0	7.8	0.7	0.3
TS	503±2	70±3	83±0	13±0	330±5	7.1	0.6	0.5
TS80-02	517±4	56±1	49±0	6±0	372±5	12.2	0.8	0.5
TS80-03	521±6	65±1	57±0	8±0	350±5	10.8	0.7	0.5
TS80-04	605±0	72±0	81±0	15±0	227±0	8.8	0.7	0.3

[a] On a moisture- and ash-free basis

The C, H, N and S contents of HAs from CS- and TS-amended soils are larger and the O content and C/N ratio are smaller than those of SO-HA, and tend to increase and decrease, respectively, with increasing the number of SS applications. Further, SS-amended soil HAs feature an acidic functional group composition that is intermediate between that of SO-HA and SS-HAs.

The E_4/E_6 ratio of CS-HA is higher than those of SO-HA and TS-HA, which are similar. Amended soil HAs show E_4/E_6 ratios similar to or slightly higher than that of SO-HA. These results confirm that composting is more efficient than thermal drying in producing a HA more similar to native soil HA.

Table V.4 Acidic functional group contents and E_4/E_6 ratios (ratio of absorbances at 465 nm and 665 nm) of humic acids isolated from the unamended soil (SO), composted sewage sludge (CS), thermally-dried sewage sludge (TS) and soils amended with CS or TS at a rate of 80 t ha^{-1} y^{-1} for 1, 2 and 3 years (CS80-02, CS80-03, CS80-04 and TS80-02, TS80-03 and TS80-04, respectively).

Origin of HA sample	Total acidity	COOH	Phenolic OH	E_4/E_6 ratio
	(mmol g^{-1})[a]			
SO	5.23	3.30	1.94	5.1
CS	3.94	2.58	1.36	6.2
CS80-02	4.22	2.83	1.39	5.1
CS80-03	4.99	3.37	1.61	6.6
CS80-04	5.16	3.50	1.66	5.9
TS	3.05	2.40	0.65	4.9
TS80-02	4.61	3.15	1.46	5.4
TS80-03	3.86	2.56	1.30	4.9
TS80-04	4.12	2.87	1.26	5.5

V.3.2.2. FT IR spectra

The FT IR spectra of HAs isolated from CS, and especially TS, differ markedly from that of SO-HA (**Figure V.1**). The main features of these spectra and their corresponding assignments according to Bellamy (1975), MacCarthy and Rice (1985) and Stevenson (1994), are the following: (a) an intense broad band between 3390 cm^{-1} and 3405 cm^{-1}, which is usually attributed to O-H stretching and, secondarily, to N-H stretching of various functional groups; (b) two sharp bands at about 2925 cm^{-1} and 2855 cm^{-1} due to aliphatic C-H stretching, whose relative intensity is much stronger in TS-HA and in HAs from TS-amended soils than in SO-HA, CS-HA and HAs from CS-amended soils; (c) a band between

1631 cm^{-1} (SO-HA) and 1653 cm^{-1} (TS-HA), generally attributed to absorptions of several groups including aromatic C=C, C=O stretching of amide groups (amide I band), quinonic C=O and/or C=O of H-bonded conjugated ketones; (d) a strong peak at 1540 cm^{-1}, present only in TS-HA and preferentially ascribed to N-H deformation and C=N stretching of amides (amide II band); (e) a weak peak at 1456 cm^{-1} in TS-HA, which is only a shoulder in all other HAs, generally attributed to aliphatic C-H deformation; (f) a common band of medium intensity between 1416 cm^{-1} and 1380 cm^{-1}, possibly due to C-H deformation of CH_2 and CH_3 groups and/or to antisymmetric stretching of COO^- groups; (g) a band centred at about 1231-38 cm^{-1}, which is strong in TS-HA and weak or a shoulder in SO-HA and CS-HA, preferentially ascribed to C-O stretching of aryl ethers; and (h) an absorption at about 1045-40 cm^{-1} generally attributed to C-O stretching of polysaccharide-like substances, whose relative intensity is very strong in TS-HA, much weaker in CS-HA and amended soil HAs, and a shoulder in SO-HA. These results confirm the greater aliphatic character and larger content of N-containing groups, most probably polypeptidic chains, and polysaccharide components of TS-HA, with respect to CS-HA, and especially SO-HA. In general, CS-HA and TS-HA appear to have incorporated proteinaceous materials and aliphatic constituents, like fatty acids and waxes, which are typical in raw SS (Boyd et al., 1980; García-Gil et al., 2004; Adani and Tambone, 2005).

The FT IR spectra of HAs isolated from amended soils differ from that of SO-HA in featuring an increase of the relative intensities of absorptions of aliphatic structures, amide groups and polysaccharide-like components. These effects are more evident in HAs from TS-amended soils than in HAs from CS-amended soils, especially with increasing the number of amendments. In conclusion, FT IR results indicate that amendment with CS, and especially with TS, affect partially the structure and functional properties of amended soil HAs, possibly due to the partial incorporation of HA-like fractions from CS and TS into native soil HA.

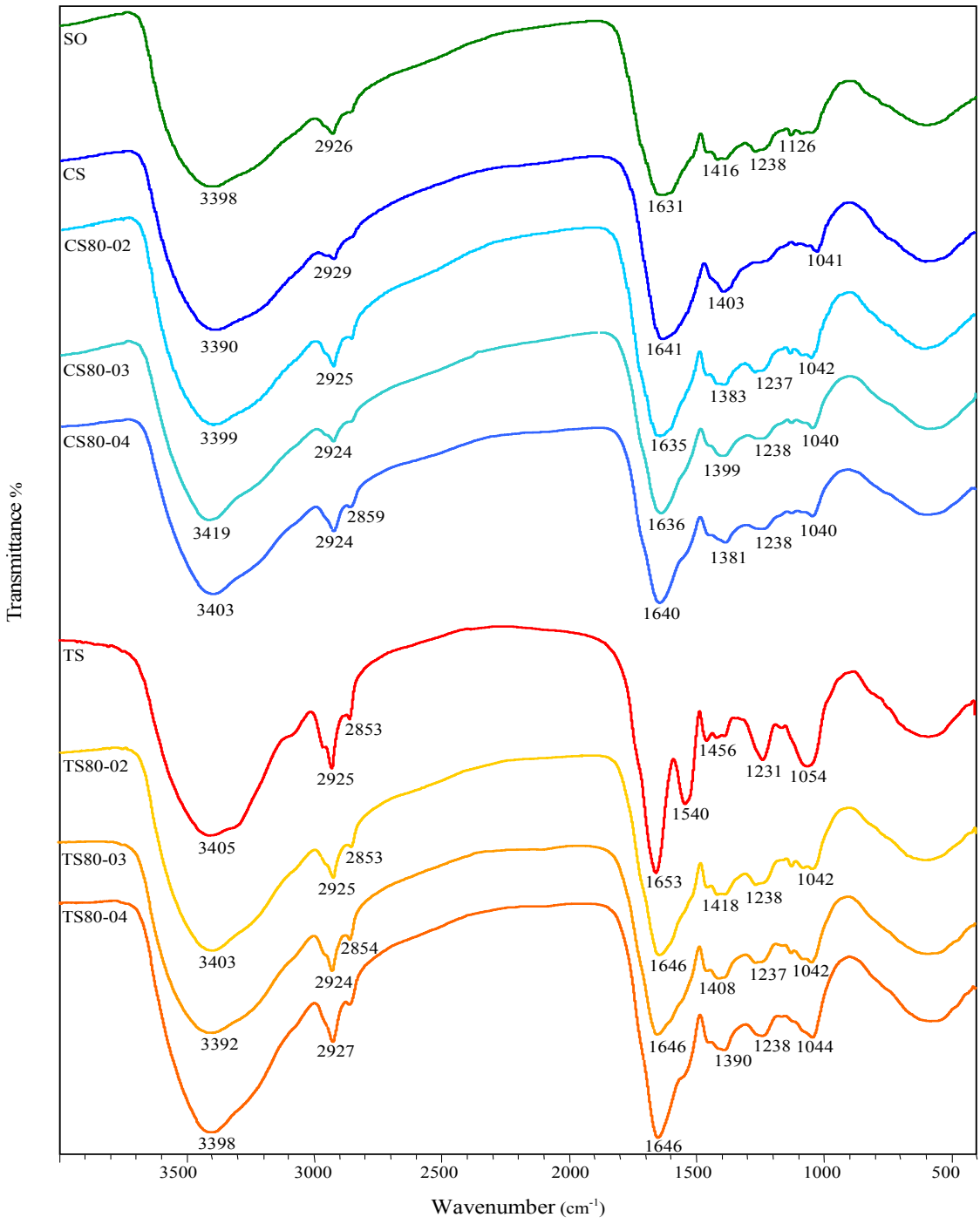

Figure V.1: Fourier transform infrared (FT IR) spectra of humic acids isolated from the unamended soil (SO), composted sewage sludge (CS), thermally-dried sewage sludge (TS) and soils amended with CS or TS at a rate of 80 t ha^{-1} y^{-1} for 1, 2 and 3 years (CS80-02, CS80-03, CS80-04 and TS80-02, TS80-03 and TS80-04, respectively).

V.3.2.3. Fluorescence spectra

The monodimensional fluorescence spectra in the emission, excitation, and synchronous-scan modes of the HAs examined are shown in **Figures V.2**, **V.3** and **V.4** respectively, whereas the relative fluorescence intensity values from the emission spectra (RFI_{em}) are listed in **Table V.5**. The SO-HA features a typical emission broad band with the maximum positioned at 459 nm, and a shoulder at about 498 nm, whereas CS-HA and TS-HA show the broad emission band centred at 439 nm and 438 nm, respectively, which, in the case of CS-HA, is accompanied by a long tail that extends towards longer wavelengths. The peak at 410 nm in the TS-HA emission spectrum is attributed to the Raman band of water (Senesi, 1990). With increasing the number of CS, and especially TS, additions, the emission maximum in amended soil HAs shifts toward shorter wavelengths. The RFI_{em} value of CS-HA is slightly lower than that of TS-HA, and both are much smaller than RFI_{em} of SO-HA (**Table V.4**). As an effect of SS addition, the RFI_{em} values of HAs from amended soils tend to decrease progressively with the number of SS additions, with respect to that of SO-HA.

The excitation spectrum of SO-HA is characterized by two major peaks at 441 and 452 nm and two less intense peaks at 396 and 412 nm. The excitation spectrum of CS-HA features two intense peaks at intermediate (400 nm) and long (441 nm) wavelengths, while TS-HA shows a main peak at 355 nm and a minor peak at 440 nm. The shape of excitation spectra of amended soil HAs resemble that of SO-HA but the relative intensities of the peaks at long wavelength (441 and 452 nm) decrease with respect to that of the peaks at intermediate and short wavelengths with increasing the number of amendments.

The synchronous scan spectrum of SO-HA is characterized by a prominent peak at 465 nm and a much weaker peak at 416 nm, whereas the spectra of HAs from CS and TS feature a prominent peak at short wavelength (391 nm and 381 nm, respectively), which is much more intense in TS-HA, and a less intense peak at intermediate wavelength (440 nm and 434 nm, respectively). The shape of the synchronous scan spectra of amended soil HAs resembles that of SO-HA, but exhibit an evident decrease of the relative intensity of the major peak with increasing the number of SS additions.

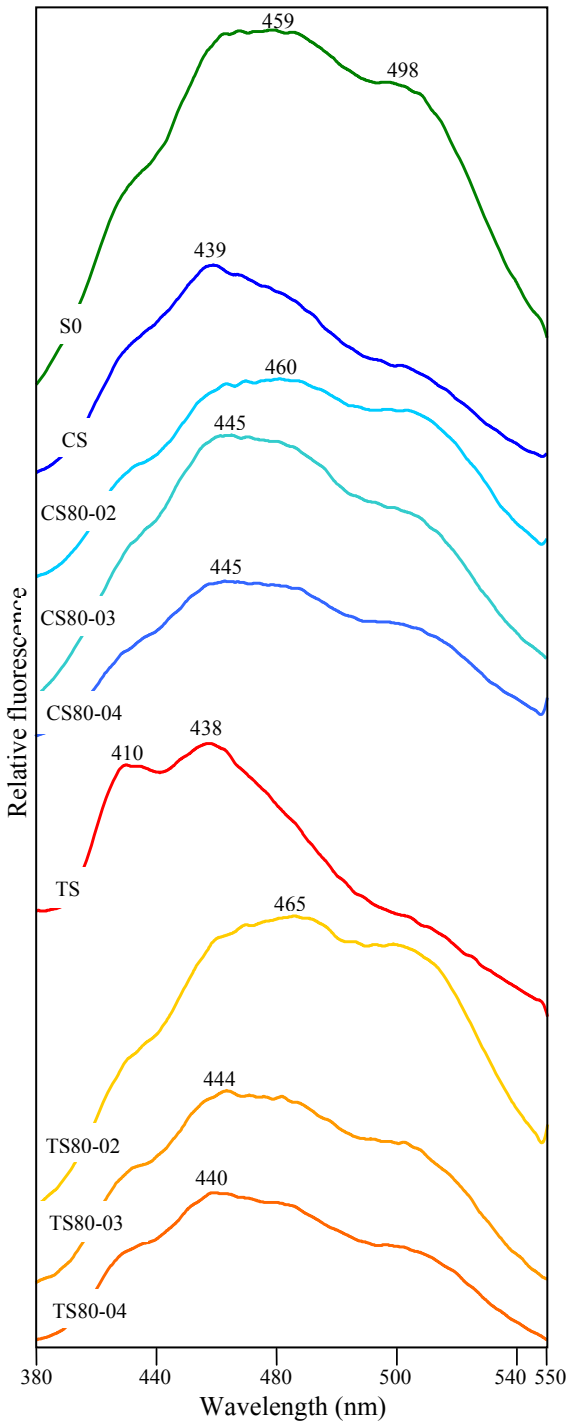

Figure V.2: Fluorescence emission spectra of humic acids isolated from the unamended soil (SO), composted sewage sludge (CS), thermally-dried sewage sludge (TS) and soils amended with CS or TS at a rate of 80 t ha^{-1} y^{-1} for 1, 2 and 3 years (CS80-02, CS80-03, CS80-04 and TS80-02, TS80-03 and TS80-04,respectively).

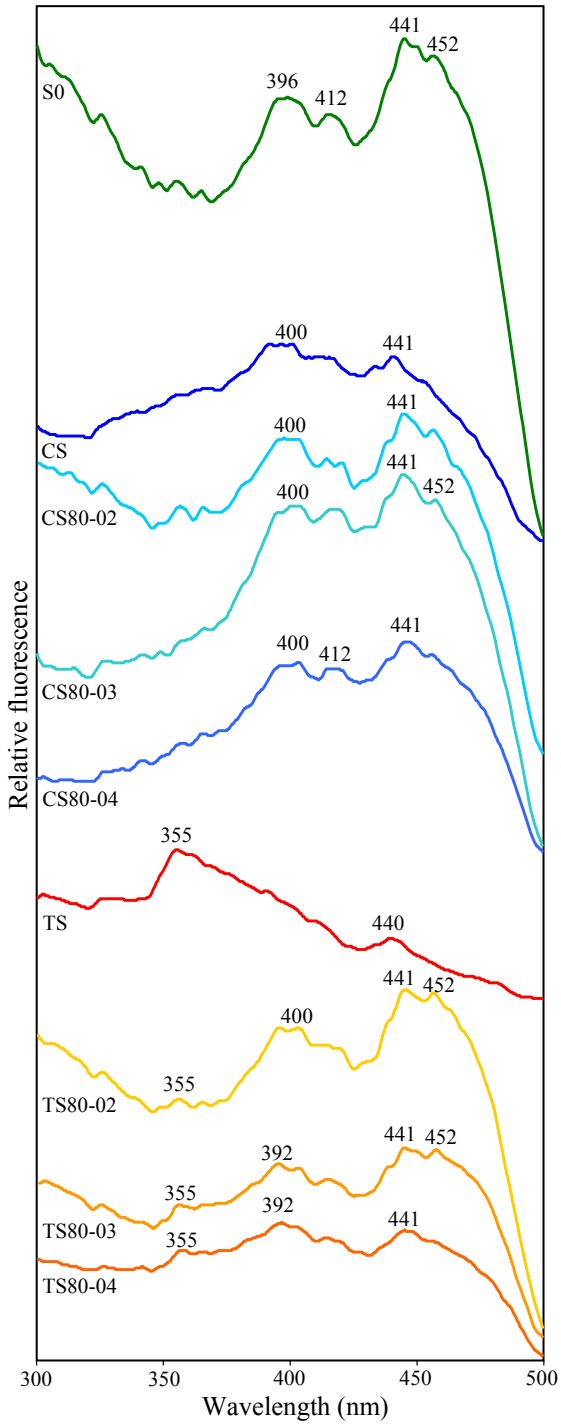

Figure V.3: Fluorescence excitation spectra of humic acids isolated from the unamended soil (SO), composted sewage sludge (CS), thermally-dried sewage sludge (TS) and soils amended with CS or TS at a rate of 80 t ha^{-1} y^{-1} for 1, 2 and 3 years (CS80-02, CS80-03, CS80-04 and TS80-02, TS80-03 and TS80-04, respectively).

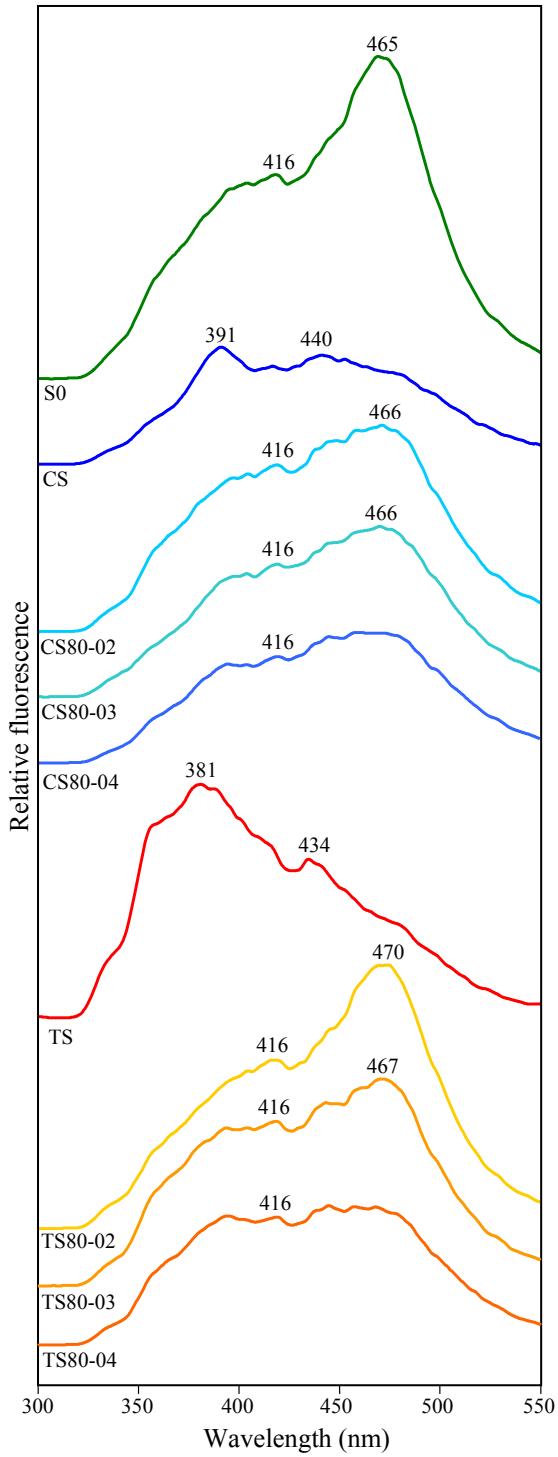

Figure V.4: Fluorescence synchronous scan espectra of humic acids isolated from the unamended soil (SO), composted sewage sludge (CS), thermally-dried sewage sludge (TS) and soils amended with CS or TS at a rate of 80 t ha^{-1} y^{-1} for 1, 2 and 3 years (CS80-02, CS80-03, CS80-04 and TS80-02, TS80-03 and TS80-04, respectively).

The fluorescence EEM spectrum of SO-HA (shown as a contour map in **Figure V.5**) features two fluorophores, the major one at an excitation/emission wavelength pair (EEWP) of 390/470, and the minor one at EEWP = 506/440. The EEM spectra of CS-HA and TS-HA consist of a unique main band centred at a similar EEWP (340/438 and 340/437, respectively). Similar to RFI_{em} values, the relative fluorescence intensity values from the EEM spectra (RFI_{EEM}) of the main peak of TS-HA (**Table V.5**) is slightly higher than that of CS-HA, and both are smaller than that of SO-HA. The EEM spectra of HAs from soils amended once with CS and TS are similar to that of SO-HA, whereas those of HAs from soils amended for 2 and 3 years feature a unique fluorophore. With increasing number of TS additions, the EEWP of this fluorophore tends to shift to shorter values in the case of TS-amended soil HAs, and the RFI_{EEM} values of CS- and TS-amended soil HAs decrease progressively.

Table V.5: Emission wavelength (EW) and relative fluorescence intensity values (RFI_{em}, arbitrary units) calculated from the emission spectra and excitation/emission wavelength pairs (EEWP; nm/nm) and relative fluorescence intensity (RFI_{TL}, arbitrary units) of the main peak in total luminescence spectra of humic acids isolated from the unamended soil (SO), composted sewage sludge (CS), thermally-dried sewage sludge (TS) and soils amended with CS or TS at a rate of 80 t ha^{-1} y^{-1} for 1, 2 and 3 years (CS80-02, CS80-03, CS80-04 and TS80-02, TS80-03 and TS80-04, respectively).

Origin of HA sample	EW	RFI_{em}	EEWP	RFI_{TL}
SO	459	245	390/470	258
CS	439	134	340/438	147
CS80-02	461	148	390/469	158
CS80-03	445	168	390/468	180
CS80-04	443	106	395/467	113
TS	438	181	340/437	201
TS80-02	466	199	440/508	219
TS80-03	444	151	355/444	154
TS80-04	439	113	355/444	118

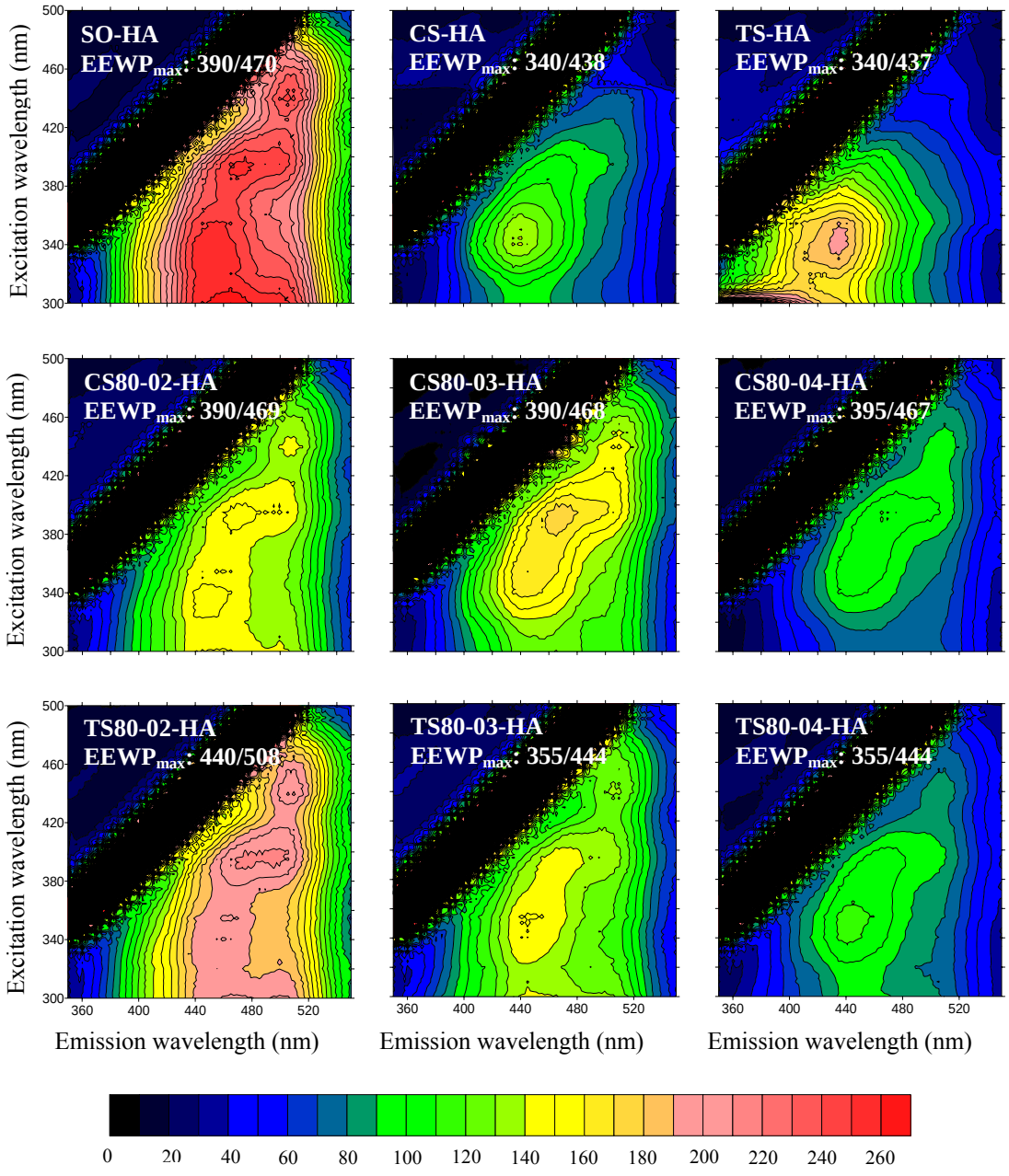

Figure V.5: Fluorescence excitation-emission matrix (EEM) spectra (contour maps) of humic acids isolated from the unamended soil (SO), composted sewage sludge (CS), thermally-dried sewage sludge (TS) and soils amended with CS or TS at a rate of 80 t ha^{-1} y^{-1} for 1, 2 and 3 years (CS80-02, CS80-03, CS80-04 and TS80-02, TS80-03 and TS80-04, respectively).

In general, fluorescence features of SS-HAs and soil HAs are similar to those obtained previously by other authors for HAs of similar origin (Mobed et al., 1996; Soler-Rovira et al., 2003, Plaza et al., 2006a). The short wavelength of the main fluorescence peak and the small fluorescence intensity of CS-HA and TS-HA suggest the presence of simple structural components of wide molecular heterogeneity and small molecular size, low degree of aromatic polycondensation, low level of conjugated chromophores, and low humification degree (Senesi et al., 1996, Soler-Rovira et al., 2002, Plaza et al., 2006a). On the contrary, the long wavelength and large fluorescence intensity of the major peak of SO-HA may be ascribed to the presence of an extended, linearly-condensed aromatic ring network and other unsaturated bond systems capable of a great degree of conjugation in large molecular size and extensively humified macromolecules. Further, the fluorescence behaviour of amended soil HAs suggests a partial incorporation of simple and low-humified components of SS-HA into native soil HA, which is more evident in HAs from TS-amended soils.

V.4. CONCLUSIONS

Substantial differences exist in the chemical composition, structure and functionalities of CS-HA and TS-HA. In particular, CS-HA features a more polycondensed, oxidized, unsaturated and aromatic structure, with respect to TS-HA that shows a prevalent aliphatic character and a larger content of polysaccharides and proteinaceous components distinctive of a less mature organic material. As compared to native soil HA, CS-HA and TS-HA feature a lower degree of aromatic ring polycondensation, polymerytization and humification. Consecutive additions of TS affect the compositional, structural and functional characteristics of soil HAs more markedly than CS.

VI. Effects of Long - Term Soil Amendment With Sewage Sludges on Soil Humic Acid Thermal and Molecular Properties

VI. EFFECTS OF LONG-TERM SOIL AMENDMENT WITH SEWAGE SLUDGES ON SOIL HUMIC ACID THERMAL AND MOLECULAR PROPERTIES

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INDEX

ABSTRACT

Sewage sludges are frequently used as soil amendments due to their high contents of organic matter and nutrients, particularly N and P. However, their effects upon the chemistry of soil humic acids, one of the main components of the soil organic matter, need to be more deeply studied. Sewage sludges subjected to two different types of pre-treatment (composted and thermally dried) were applied for 3 consecutive years to an agricultural soil under long-term field study. Thermal analysis (TG-DTG-DTA) and solid-state ^{13}C NMR spectroscopy were used to determine changes and compare molecular and structural properties of humic acid samples isolated from sewage sludges, unamended, and amended soils. Results showed important differences in the chemical composition and structure of the two types of humic acids from sewage sludges, with an important presence of alkyl and N-alkyl compounds in the thermally dried sewage sludge humic acids. After long-term application of both SS, soil humic acids, that originally presented a marked aromatic character, exhibited enrichment in aliphatic and proteinaceous constituents.

Keywords: Sewage sludge; Humic acids; ^{13}C NMR; Thermal analysis.

VI.1. INTRODUCTION

Animal manure and crop residues have traditionally been applied to soil as a means of maintaining and increasing the organic matter content. However, nowadays their production cannot satisfy the current soil requirements of cropland. This has motivated the growing interest in agricultural applications of organic residues derived from a wide variety of human activities as a convenient alternative to their landfill disposal (Provenzano et al., 1998; Fernández et al., 2007b). In this context, sewage sludge (SS), the residual solid produced during wastewater treatment, has been increasingly applied as an organic amendment due to its high content of organic matter and nutrients, particularly N and P (Smernik et al., 2003).

However, before being used, as other organic waste materials, SS must be properly processed in order to obtain a mature, stabilized organic matter and avoid possible detrimental effects of their premature application (Senesi et al., 1996; Plaza et al., 2003). Composting is one of the most common treatments that resembles natural processes in soil, being an accelerated humification process (Senesi, 1989; Provenzano et al., 1998). Another relatively new treatment method is thermal-drying that consists of a short and intense heating of the SS, destroying pathogens, eliminating volatile chemicals and leading to a sanitized final product with good handling characteristics but lacking a maturation process (Fernández et al., 2007b).

Although beneficial effects upon the physical (Navas et al., 1998; Sort and Alcañiz, 1999), chemical (Navas et al., 1998), and biological properties (Banerjee et al., 1997; Wong et al., 1998) have been widely investigated, little information is available on long-term effects induced by SS on soil humic substances, and particularly on humic acids (HAs), the most abundant and chemically significant fraction of soil organic matter (Senesi, 1989; Reveille et al., 2003, Adani and Tambone, 2005). Due to the important role that HAs play in the soil, information about their properties and transformations can help in understanding and evaluating the use of SS as organic amendments.

Among the numerous chemical and spectroscopic methods used to study HA structures and composition, thermal investigations are steadily gaining interest. However, most of the thermal studies are still related to characterizations of HAs from soils (Gonet and Cieslewicz, 1998; Dell'Abate et al., 2002) or composting materials (Provenzano et al., 1998; Pietro and Paola, 2004), and do not focus on the evolution of HAs in soil organic matter restoration experiments.

The use of solid-state ^{13}C nuclear magnetic resonance (NMR) spectroscopy has emerged as one of the most useful and powerful tools to obtain structural information of humic substances (Wilson, 1987; Hatcher et al., 2001; Smernik et al., 2003). The combination of cross polarization (CP) and magic angle spinning (MAS) is the most popular ^{13}C NMR technique for the study of humic materials (Conte et al., 1997; Cook and Langford, 1998; Chefetz et al., 2002). However, this technique can underrepresent some structural units (e.g., fused aromatic moieties, alkyl groups with high degrees of molecular mobility, and carbonyl C) (Dria et al., 2002; Smernik et al 2003). These studies, in combination with our own previous experiences, have suggested the use of the alternative Bloch decay (BD) technique in the analysis of C-rich HAs. Although this technique generally has longer requirements of time and lower signal to noise ratio than the CP, the BD provides the most accurate quantitative distributions of structural units in humic acids (Smernik and Oades, 2000; Dria et al., 2002).

In this study, solid-state ^{13}C NMR and thermal analyses (TG, DTG and DTA) were performed in order to: (a) determine and compare molecular and structural properties of the HA isolated from composted sewage sludge (CS) and thermally-dried sewage sludge (TS), and (b) investigate their contributions to the soil-HA in over three years.

VI.2. MATERIALS AND METHODS

VI.2.1. Sewage sludges, soil and field experiment

The CS sample was collected from a 3-month windrow-composted mixture of three SS originated from three municipal wastewater treatment plants in Madrid (Spain) metropolitan area. The CS had the mean composition: dry matter content, 435.8 g kg^{-1}; pH, 7.1; electrical conductivity, 3.90 dS m^{-1}; total N content, 23.9 g kg^{-1}; and total organic C, 181.0 g kg^{-1}. The TS sample consisted of a SS dried with hot air at temperatures between 380ºC and 450ºC in the wastewater treatment plant "SUR" in Madrid metropolitan area. The TS had the mean composition: dry matter content, 846.4 g kg^{-1}; pH, 7.0; electrical conductivity, 1.5 dS m^{-1}; total N content, 35.6 g kg^{-1}; and total organic C, 296.0 g kg^{-1}.

The field experiment was conducted in the experimental farm "La Higueruela" located in Toledo (Spain). The soil is a Calcic Luvisol (FAO, 1998) or Typic Haploxeralf (Soil Survey Staff, 2003), having a sandy loam texture (sand, 590 g kg^{-1}; silt, 220 g kg^{-1}; clay, 190 g kg^{-1}); pH, 5.7; electrical conductivity, 0.05 dS m^{-1}; total N content, 0.9 g kg^{-1}; and total organic C, 7.2 g kg^{-1}. The site is characterized by a continental semiarid climate with an average annual rainfall of about 487 mm and an average annual temperature of 14 ºC.

The experimental design consisted in four random blocks of nonirrigated soil plots (10 x 3 m^2) cropped with barley (*Hordeum vulgare* L.), either unamended (SO) or amended yearly over a 3-year period at a rate of 80 t ha^{-1} with CS (CS80) or TS (TS80). Both SS were applied in mid-September, prior to barley planting in mid-October, and immediately incorporated into soil at a depth of 0-15 cm. The third year, after barley harvesting, in late June, four surface soil subsamples (Ap horizon, 0-15 cm depth) were collected randomly from each plot. Each soil subsample consisted of a mixture of 20 soil cores each of 3-cm diameter. A composite sample was then obtained for each treatment by mixing equal amounts (1 kg) of the four corresponding soil subsamples.

VI.2.2. Humic acid isolation

The HA fractions were isolated from CS, TS, CS80, TS80, and SO samples by conventional methods. Prior to extraction of HA, soil and SS samples were air-dried and passed through a 2 mm sieve after removal of plant residues and stones from soils.

Carbonates were removed from soil samples by mechanical stirring with 2M H_3PO_4 for 30 min. The treatment was repeated three times, and then the samples were washed with distilled water until the suspension reached a pH of 7. Carbonate-free soils and SS samples were first extracted with 0.1 M $Na_4P_2O_7$ at pH 9.8 and then by 0.1 M NaOH, at room temperature (RT, about 293 K), and using a sample to extractant ratio of 1:10. Each extraction was repeated three times by mechanically shaking the mixture for 3 h, then centrifuging at 15300 x g for 15 min, and filtering the supernatant through a Whatman no. 31 filter paper. The combined alkaline supernatants were then acidified with HCl until reaching a pH=1, left standing for 24 h in a refrigerator to allow the complete precipitation of HA, centrifuged at 30100 x g for 15 min, and then filtered through a Whatman no. 31 filter paper. The HA precipitates were then purified by dissolution in 0.1 M NaOH, centrifugation at 30100 g, elimination of the residue, acidification of the alkaline supernatant with HCl-HF until pH 1, and standing for 12 h in a refrigerator. The purification procedure was repeated three times. The precipitated HAs were then recovered with distilled water, dialyzed until free of Cl^- ions, and finally freeze-dried.

VI.2.3. Humic acid characterization

The moisture content of HAs was measured by heating overnight at 105 ºC and ash content by combustion overnight at 550 ºC. The C, H, N and S contents were determined in triplicate using a Fisons Instruments (Crawley, UK) elemental analyser model EA 1108. Oxygen content was calculated by difference on an ash-free dry weight basis as: O%= 100 – (C+H+N+S) %.

Thermogravimetry (TG), differential thermogravimetry (DTG) and differential thermal analysis (DTA) were performed simultaneously using a Setaram (Caluire, France) SETSYS thermal analyzer equipped with the computer program Setsoft 1.6.4 (Setiram, Caluire, France) for data processing. Ten-milligram HS samples were isothermally heated from room temperature to 30 ºC for 10 min and then to 1200 ºC in a platinum crucible under a flow of air of 30 mL min^{-1}. The heating rate was set at 10 ºC min^{-1}. Calcined alumina was used as reference material.

Solid-state ^{13}C NMR experiments were performed on a Bruker (Billerica, MA) DMX 300 NMR spectrometer. This spectrometer operates at a ^{13}C frequency of 75.48 MHz. Samples were placed in a 4 mm (outside diameter) NMR rotor with a Kel-F cap (3M, Minneapolis, MN). Bloch decay spectra were acquired using a 20-degree ^{13}C pulse, a recycle delay of 3 s, and an MAS frequency of 13 kHz. The recycle delay was calculated for a 20 degree ^{13}C pulse using the Ernst Anderson equation, based upon a 30 s T_1 relaxation time following a 90 degree pulse (Ernst and Anderson, 1966). Around 22500 scans were collected for each sample. The Kel-F rotor caps and probe components give rise to a significant ^{13}C signal in the BD spectrum, which overlapped with the NMR signal of the samples. To correct for this, the empty-rotor spectrum was acquired separately and was subtracted from each BD spectrum before integration. Forty-hertz line broadening was applied when fitting the NMR spectra and peak areas were integrated according to the chemical shift regions defined in **Table VI.3**. Spin counting experiments were performed using the method of Smernik and Oadeds (2000). Cellulose (Sigma Aldrich Co.) was used as an external intensity standard (i.e., the cellulose spectrum was acquired separately to those of the samples).

VI.3. RESULTS AND DISCUSSION

VI.3.1 Elemental analysis

The elemental composition of CS-HA (**Table VI.1**) features higher O/C and lower H/C and N/C ratios than TS-HA. These results suggest that composting favours the formation of a more oxidized HA fraction than thermal drying. This is typical of a more mature organic material (Senesi et al., 1996). Similar results for composted and uncomposted SS are reported by other authors (Boyd et al., 1980; Gerasimowicz and Byler, 1985).

Relative to SO-HA, CS-HA and TS-HA have lower or much lower C, O contents, and larger or much larger N and S contents. The high levels of N and S in SS-HAs may be ascribed to the incorporation of proteinaceous products and S-containing commercial surfactant residues in the HA fraction (Senesi et al., 1996). In comparison with SO-HA, the

C, H, N and S contents of HAs from both SS-amended soils have increased due to partial incorporation of compounds from CS and TS into soil HA.

Table VI.1: **Moisture, ash content, elemental composition and atomic ratios (± standard error) of humic acids (HA) isolated from the unamended soil (SO), composted sewage sludge (CS), thermally-dried sewage sludge (TS) and soils amended with CS or TS at a rate of 80 t ha^{-1} y^{-1} for 3 years (CS80 and TS80, respectively).**

Origin of HA sample	Moisture	Ash	C	H	N	S	O	C/N	H/C	O/C
	(g kg^{-1})		(g kg^{-1})[a]					(Atomic ratios)		
SO	69.8	79.2	505±6	53±3	44±1	4±0	394±9	13.3	1.3	0.6
CS	35.9	115.7	460±1	53±1	85±0	14±0	389±1	6.3	1.4	0.6
CS80	24.9	61.2	598±0	67±0	90±0	16±0	228±0	7.8	1.4	0.3
TS	15.9	40.5	503±2	70±3	83±0	13±0	330±5	7.1	1.7	0.5
TS80	40.4	84.3	605±0	72±0	81±0	15±0	227±0	8.8	1.4	0.3

[a] On a moisture- and ash-free basis

VI.3.2. Thermal analysis

Thermograms of HA samples are shown in **Figure VI.1** and thermal main parameters are summarised in **Table VI.2**. The HA samples from TS reveal a significantly different thermal behaviour than CS-HA and SO-HA, which are fairly similar. Meanwhile, HA samples from SS amended soils are quite similar. The primary features of the differential thermograms are: (a) a small endothermic peak at low temperatures (90.0 – 101.8 ºC), ascribed to reactions of dehydration; (b) an exothermic peak within the 338-341 ºC range, generally attributed to the degradation of polysaccharides, decarboxylation of acidic groups and dehydration of aliphatic alcohols (Flaig et al., 1975; Dell'Abate et al., 2002; Francioso et al., 2005); (c) an exothermic peak between 439ºC and 456ºC, possibly due to the presence of N compounds (e.g. proteinaceous material) and long chain hydrocarbons (Francioso et al., 2005); and (d) an exothermic peak at high temperatures (539 - 560 ºC), usually related to the combustion of aromatic structures and cleavage of C-C bonds (Peuravuori et al., 1999).

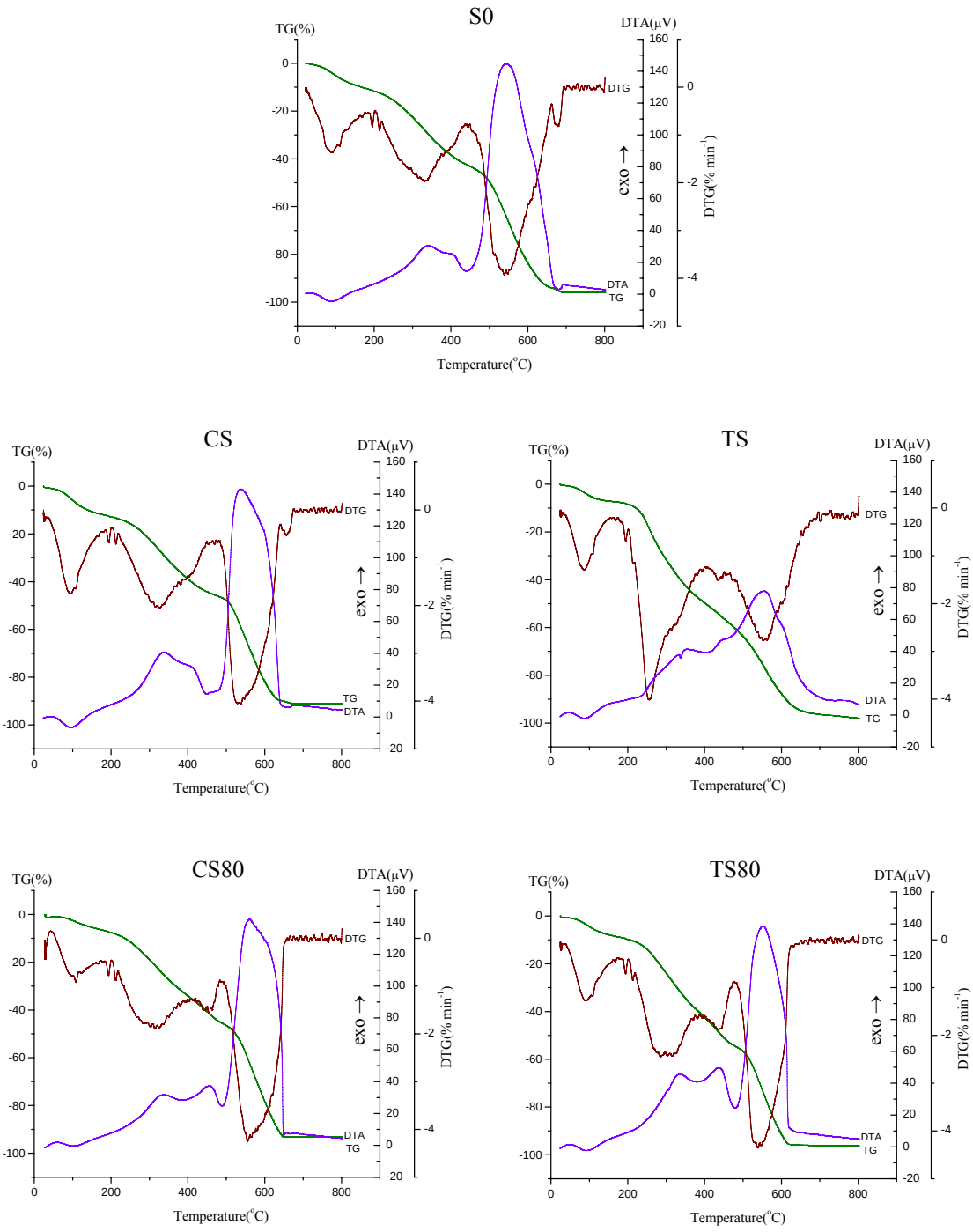

Figure VI.1: TG, DTG and DTA thermograms of humic acids (HA) isolated from the unamended soil (SO), composted sewage sludge (CS), thermally-dried sewage sludge (TS) and soils amended with CS or TS at a rate of 80 t ha^{-1} y^{-1} for 3 years (CS80 and TS80, respectively).

Exothermic reactions, which determine weight losses at different steps recorded on the TG curves, allow the quantitative comparison of the different fractions involved in the reactions (**Table VI.2**). For the first exothermic reaction, CS-HA shows a lower weight loss than TS-HA, which features the most important lost of weight. The DTG curves in this region also indicate the rapid combustion of the TS-HA compounds at this range of temperatures in comparison with the other samples. These results suggest the presence of a large proportion of carbohydrates and aliphatic alcohols structures in TS-HA (Ricca et al., 1993). The exothermic peak at 439ºC-456ºC, unique to the HA from SS-amended soils, is slightly higher for the TS80-HA than for CS80-HA. This is consistent with the persistence of proteinaceous and/or long chain N-containing surfactant structures present in the TS-HA (8.1% weight loss), although the presence of N-aromatic compounds (e.g. melanoidin) due to the condensation of reducing sugars and amines, must be also considered (Stevenson, 1994). The last exothermic peak features slightly higher loss of weight for CS-HA (45.3%) in comparison with TS-HA (38.2%). However the biggest mass loss (53.1%) corresponds to SO-HA. Further, SO- and CS-HA show a higher decomposition rate than TS-HA. This could be attributed to the presence of aromatics in CS-HA and SO-HA (Sheppard and Forgeron, 1987; Francioso et al., 2005). This is confirmed by ^{13}C NMR, as discussed in section VI.3.3.

VI.3.3 Bloch decay ^{13}C nuclear magnetic resonance analysis

The description of the chemical nature of the organic C contained in HA is more directly conferred by analysis of the BD ^{13}C NMR spectra (**Figure VI.2**). Spectra were divided into four regions and integrated as follows: 0 to 45 ppm assigned to aliphatic C; 45 to 110 ppm assigned primarily to O-substituted alkyl C in carbohydrates, but also including methoxyl C and N-substituted alkyl C in protein; 110 to 160 ppm assigned to aryl C, including O-aryl and C- and H- substituted aryl C; and 160 to 220 ppm assigned to carbonyl C in carboxylic acids, ketones, esters, and amides (**Table VI.3**). Spin counting experiments confirm that 90-100% of the HA-C was observed in the BD spectra for all the samples analysed.

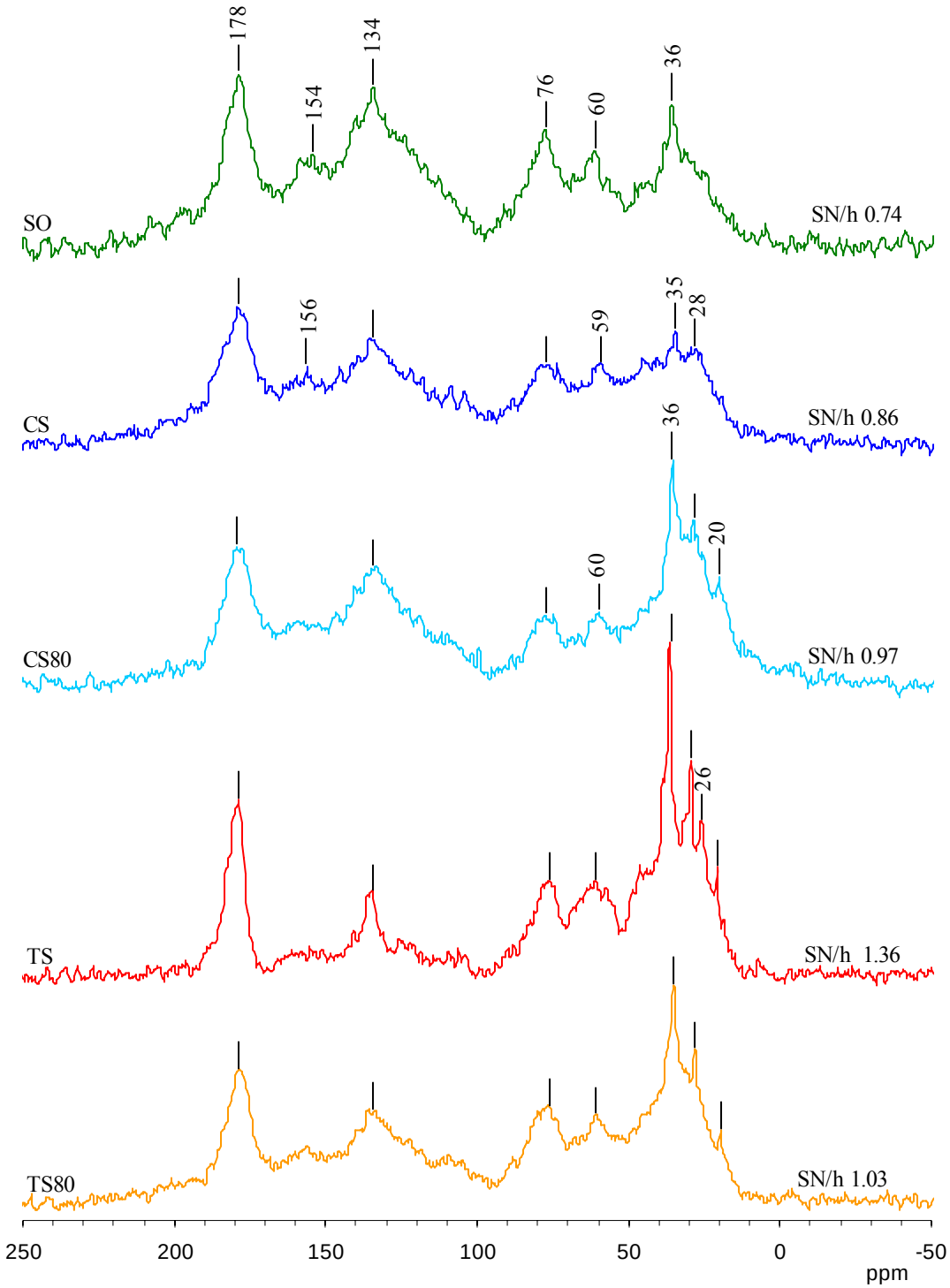

Figure VI.2: Bloch Decay ^{13}C NMR spectra of humic acids (HA) isolated from the unamended soil (SO), composted sewage sludge (CS), thermally-dried sewage sludge (TS) and soils amended with CS or TS at a rate of 80 t ha^{-1} y^{-1} for 3 years (CS80 and TS80, respectively). SN/h is the signal-to-noise ratio per hour of acquisition time.

Humic acids extracted from TS and CS show a higher content of the aliphatic and O/N-alkyl C and a lower content of aromatic and carbonyl/amide C than SO-HA. These differences are more marked for ^{13}C NMR spectra of HA extracted from TS, which is dominated by the aliphatic resonance area at d<45 ppm with different peaks. In particular, signals at d=20 ppm indicate methyl C in alkyl chains, and the signal at 28 ppm represents methylene C in long alkyl chains. Meanwhile, the strong signal at 36 ppm is due to branched alkyl chains, such as R-CH and R-CH_2 a substituted groups where R=COOH (Ricca et al., 2000). In contrast, the region between 110 and 160 ppm is predominant for SO-HA and for CS-HA, although lower in the case of CS-HA, showing the abundance of aromatic C (e.g. peak at 134 ppm.) and O-substituted aromatic C (e.g. peak at 154 ppm.) (Chefetz et al., 2002). These results agree with elemental and thermal analyses. The existence of a large proportion of alkyl and O/N alkyl structures in the TS-HA could be attributed to the lower degree of a maturation of the TS organic matter. Similar results have been reported for raw SS in other studies (Smernik et al., 2003; Adani and Tambone, 2005). Meanwhile, the composting process to which CS has been submitted has resulted in HA with more recalcitrant structures (i.e. thermally stable compounds) rather similar to that of SO-HA.

Table VI.3: Bloch Decay ^{13}C NMR relative area (%) for humic acids (HA) isolated from the unamended soil (SO), composted sewage sludge (CS), thermally-dried sewage sludge (TS) and soils amended with CS or TS at a rate of 80 t ha^{-1} y^{-1} for 3 years (CS80 and TS80, respectively).

HA sample	Aliphatic-C (0<δ≤45 ppm)	O/N-Aliphatic-C (45<δ≤110 ppm)	Aromatic-C (110<δ≤160 ppm)	Carboxyl/Amide-C/Ketonic-C (160<δ≤220 ppm)
			%	
SO	16.8	25.8	34.5	23.0
CS	22.0	27.7	28.5	21.7
CS80	32.6	20.8	28.6	17.9
TS	38.5	30.8	14.3	16.4
TS80	30.1	28.7	23.8	17.4

After three years of consecutive additions, HA from soils amended with both SS clearly differ from those of the original unamended soil. In particular, with respect to the SO-

HA, the proportion of aliphatic compounds has increased and aromatic and carbonyl and/or amide C compounds have decreased in both CS80-HA and TS80-HA. Meanwhile, the O/N-alkyl C decreased in CS80-HA and increased in TS80-HA (**Table VI.3**).

The increase in aliphatics (13.3% in TS80-HA and 15.8% in CS80-HA) constitutes the most significant contribution of both SS to soil-HA. The TS80-HA spectrum in this region (0-45 ppm) is similar to that obtained for CS80-HA and features broad peaks at 21, 28 and 36 ppm instead of the well-resolved peaks showed in TS-HA spectrum. Aliphatic structures in SS are thought to be more resistant to degradation (Genevini et al., 2002; Adani and Tambono, 2005). The incorporation of new aliphatic structures in CS80- and TS80-HA may be ascribed to the natural humification process in the soil. During this process, O-alkyl C has been postulated to be utilized by the microbial population in the soil, resulting in a relative increase of aromatic and alkyl structures, which play a fundamental role in the structure of humic acids (Hatcher et al., 1985; Almendros et al., 1991; Cook and Langford, 1998; Chefetz et al., 2002).

Although less important than the contribution of aliphatic-C from SS-HA to soil-HA, the incorporation of proteinaceous molecules has been also reported. To provide a more accurate measure of proteinaceous HA-C, the N-alkyl (45-60 ppm) and O-alkyl (60-90 ppm) spectral regions have been independently integrated. The HA from soils amended with CS and TS had slightly more N-alkyl C (6.1% and 8.2 %, respectively), than the unamended SO-HA (5.2 %). Therefore, in addition to the high contribution of aliphatic molecules, the contribution of N-substituted alkyl C from CS-HA, and especially from TS-HA, is evident in the SO-HA. These results agree with those found in thermal analysis as well as with the increase of H/C and N/C ratios and decreased of O contents revealed in the elemental analysis.

VI.4. CONCLUSIONS

As a whole, this work shows the benefits of using thermogravimetrics methods and NMR spectroscopy analysis in the characterization of sewage sludge and their effects on soil-HA.

Substantial differences have been observed in the chemical composition and structure of CS-HA and TS-HA. In particular, with respect to CS-HA, TS-HA features a prevalent aliphatic character and a larger content of polysaccharides and proteinaceous components, typical of a less mature organic material. The soil-HA shows a more aromatic character. After long-term application, the contributions of SS amendments to native soil are evidenced by changes in molecular composition of the soil HA. In general, soils amended with CS, and especially with TS exhibit enhancements in aliphatic and proteinaceous materials.

VII. ACID-BASE PROPERTIES OF HUMIC SUBSTANCES FROM COMPOSTED AND THERMALLY-DRIED SEWAGE SLUDGES AND AMENDED SOILS AS DETERMINED BY POTENTIOMETRIC TITRATION AND THE NICA-DONNAN MODEL

VII. ACID-BASE PROPERTIES OF HUMIC SUBSTANCES FROM COMPOSTED AND THERMALLY-DRIED SEWAGE SLUDGES AND AMENDED SOILS AS DETERMINED BY POTENTIOMETRIC TITRATION AND THE NICA-DONNAN MODEL

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INDEX

ABSTRACT

The acid-base properties of humic acids (HAs) and fulvic acids (FAs) isolated from composted sewage sludge (CS), thermally-dried sewage sludge (TS), soils amended with either CS or TS at a rate of 80 t ha^{-1} yr^{-1} for 3 years and the corresponding unamended soil were investigated by use of potentiometric titrations. The non-ideal competitive adsorption (NICA)-Donnan model for a bimodal distribution of proton binding sites was fitted to titration data by use of a least-squares minimization method. The main fitting parameters of the NICA-Donnan model obtained for each HA and FA sample included site densities, median affinity constants and widths of affinity distributions for proton binding to low and high affinity sites, which were assumed to be, respectively, carboxylic- and phenolic-type groups. With respect to unamended soil HA and FA, the HAs and FAs from CS, and especially TS, were characterized by smaller acidic functional group contents, larger proton binding affinities of both carboxylic- and phenolic-type groups, and smaller heterogeneity of carboxylic and phenolic-type groups. Amendment with CS or TS determined a decrease of acidic functional group contents and a slight increase of proton binding affinities of carboxylic- and phenolic-type groups of soil HAs and FAs. These effects were more evident in the HA and FA fractions from CS-amended soil than in those from TS-amended soil. In conclusion, composting, as compared to thermal drying, produces HA and FA fractions with acid-base properties that resemble more those typical of native soil HAs and FAs and, therefore, induce fewer modifications in amended soil HAs and FAs.

Keywords: Acid-base properties; Fulvic Acids; Humic acids; NICA-Donnan model; Proton affinity distribution; Sewage sludge; Soil amendment

VII.1. INTRODUCTION

Land application of sewage sludge (SS), a major by-product generated at wastewater treatment plants, is a worldwide agricultural practice that can provide soil benefits because of its richness in organic matter and essential plant nutrients (Clapp et al., 1994; Lundin et al., 2004). Before application to soil, raw SS is often subjected to a treatment process aimed at optimizing its efficiency and minimizing risks for the environment. Composting, which consists of a controlled biological transformation of organic matter operated by aerobic microorganisms, is a well established method to obtain chemical stabilization, biological maturation and sanitization of SS (De Bertoldi et al., 1996). Thermal drying, which consists in subjecting SS to a short and intense heating, is being increasingly introduced in wastewater treatment plants as an advanced technology. Through thermal drying SS is transformed into a dry and pasteurized granular product easy to be transported and handled (Stasta et al., 2006).

Humic substances (HS) constitute the most abundant components of soil organic matter, which is universally recognized to be essential for a healthy and productive soil. These materials are polyelectrolytes of ill-defined and structurally heterogeneous composition, various molecular weights, and consisting of aromatic and aliphatic structures associated with carboxyl, alcoholic and phenolic hydroxyl, carbonyl, amine, amide, and other functional groups. Humic acids (HAs) and fulvic acids (FAs) are the major fractions of soil HS. Despite the general structural similarity, the soil HA fraction is characterized by greater molecular weight, carbon content and aromaticity, and smaller hydrophylic character and contents of O and acidic functional groups than the FA fraction (Stevenson, 1994; Senesi and Loffredo, 1999; Hayes and Clapp, 2001; Clapp et al., 2005). Because of the presence of acidic functional groups in the molecular structure of HAs and FAs, soil HS are major players in the acid-base buffering capacity of soils and in the environmental fate, bioavailability and physico-chemical behavior of macro- and micronutrients, toxic metal ions and xenobiotic organic cations (Ritchie and Perdue, 2003; Senesi and Loffredo, 2005; Plaza et al., 2005abc, 2006b). Consequently, the effects of amendment with either composted sewage sludge (CS) or thermally-dried sewage sludge (TS) on the acid-base properties of

soil HAs and FAs is a subject of considerable interest, especially because of the risk of release of various heavy metals to soil by this agricultural practice (McBride, 2003).

The complexity of the structures and of the resulting behavior of HS has led to a variety of models to describe their interactions with protons (Tipping, 2002; Dudal and Gérard, 2004). Of particular interest is the non-ideal competitive adsorption (NICA)-Donnan model, which stands out from the others in terms of physico-chemical realism, accuracy, number of applications and incorporation in existing speciation programs (e.g., ECOSAT and Visual MINTEQ). This model addresses the binding site heterogeneity of HS by assuming a continuous distribution of proton binding sites, discriminates between chemical and electrostatic interactions, and accounts for ionic strength effects in a generic way by means of an electrostatic Donnan gel model (Koopal et al., 1994, 2001, 2005; Benedetti et al., 1995, 1996ab; Kinniburgh et al., 1996, 1999; Milne et al., 2001). By fitting the NICA-Donnan model to potentiometric titration data obtained at different ionic strengths, intrinsic (i.e., independent of pH, salt concentration or metal concentration) acid-base properties of HAs and FAs including site density, heterogeneity and proton affinity can be probed.

The objectives of this work were (a) to determine and discuss comparatively the acid-base properties of the HA and FA fractions isolated from CS and TS with respect to the unamended soil HA and FA; and (b) to investigate the effects of cumulative CS and TS applications on proton binding to soil HAs and FAs. To reach these objectives, a current potentiometric titration method and the NICA-Donnan model were used.

VII 2. MATERIALS AND METHODS

VII.2.1. Sewage sludges, soils and field experiment

The CS sample was collected from a 3-month windrow-composted mixture of three SS originated from three municipal wastewater treatment plants in Madrid (Spain) metropolitan area. The TS sample consisted of a SS dried with hot air at high temperatures (between 380ºC and 450ºC) in the wastewater treatment plant SUR in Madrid metropolitan

area. The field experiment was conducted in the experimental farm "La Higueruela" located in Toledo (Spain) on a sandy loam soil classified as a Calcic Luvisol (FAO/ISRIC/ISSS, 1998) or Typic Haploxeralf (Soil Survey Staff, 2003). The site is characterized by a continental semiarid climate with an average annual rainfall of 487 mm and an average annual temperature of 14 ºC.

The experimental design included four random blocks of non-irrigated plots (20 m × 3 m) cropped with barley (*Hordeum vulgare* L.), either unamended (S0) or amended with 80 t ha^{-1} yr^{-1} of CS or TS over a three-year period (CS80 and TS80, respectively). The CS or TS were applied in mid-September, prior to barley planting (mid-October 2001-2004), and immediately incorporated into the soil at a depth of 0-15 cm with a disk harrow. Soon after the last barley harvest (late June 2004), surface soil samples (Ap horizon, 0-15 cm depth) were collected from each plot using a soil probe of 3-cm diameter. Each soil sample consisted of a mixture of 20 soil subsamples collected randomly from each plot.

VII.2.2. Isolation of humic and fulvic acids

The HAs and FAs were isolated by a conventional procedure (Schnitzer, 1982) from CS, TS and composite soil samples obtained by mixing equal weights of the four corresponding replicates of each treatment. Briefly, a solution of 0.1 M $Na_4P_2O_7$ and 0.1 M NaOH was added to each air-dried, 2-mm sieved sample using a ratio extractant:sample = 10:1. The mixture was shaken mechanically in N_2 gas atmosphere for 24 h at room temperature (RT, 293 ± 2 K). The supernatant solution was then separated from the residue by centrifugation at 9,600 g for 30 min. The extraction procedure was repeated three times on the residue that was finally discarded. The combined alkaline supernatants were acidified with 6 M HCl to pH~1, allowed to stand for 24 h in a refrigerator to obtain the complete precipitation of HAs, and then centrifuged at 30,400 g for 15 min. The precipitated HAs were purified by repeating three times the following steps: (a) dissolution in a minimal volume of the alkaline extractant; (b) centrifugation as above; (c) removal of the residue; (d) acidification of the recovered alkaline supernatant with 6 M HCl to a pH~1; (e) standing the suspension for 24 h at RT; and (f) final centrifugation as above. The centrifuged HAs were

recovered with distilled water, dialyzed until free of Cl^- ions, and finally freeze-dried. After removal of HAs, the acidic filtrate containing the dissolved FA fraction was passed through a column of XAD-8 resin. Adsorbed FA was then recovered by elution with 0.1 M NaOH, desalted using an Amberlyst 15-cation-exchange resin, and finally freeze-dried.

VII.2.3. Potentiometric titrations

Fully automated potentiometric titrations were carried out using a Mettler Toledo (Highstown, NJ) DL77 titrator equipped with a pH electrode Mettler DG-111-SC (Highstown, NJ) that was previously calibrated with standard buffers at pH 4.00, 7.00 and 10.00. The HAs and FAs were prepared for titration by dispersing 50 mg of each freeze-dried sample in 50 mL of deionized distilled water in 100-mL thermostatic vessels. The pH was adjusted to a value of ~10.7 by addition of 0.1 M KOH, and the mixture was stirred for 1 h under N_2 gas. Hysteretic effects were eliminated by performing two successive acid titrations with 0.1 M HNO_3 until pH ~3.3 (backward titration) followed by forward titrations with 0.1 M KOH until pH ~10.7. Then four further backward and forward titrations at ionic strengths of 0.01, 0.05, 0.1 and 0.3 M (adjusted with KNO_3) were performed and considered for calculations. Acid and base titrants were dispensed using automatic syringes in aliquots of 0.002 to 0.05 mL in order to have an electrode potential variation smaller than 10 mV. Samples were maintained at a constant stirring speed, at a temperature of 298 K, and under N_2 atmosphere throughout the titrations. The volume of titrant added and pH (when stable for 30 s with a drift of no more than ± 0.02 mV) were recorded after each addition of titrant. Forward and backward titrations gave identical results. In this paper, only forward titrations are reported.

VII.2.4. Modeling proton binding

The NICA-Donnan model was used to analyze the proton binding behavior of HAs and FAs and to determine their acid-base properties. In the Donnan model, the HAs and FAs are considered to form an electrically neutral gel-like phase having a volume throughout

which a uniform, averaged electrostatic potential known as the Donnan potential (ψ_D) exists. The electroneutrality condition of the gel phase is given by

$$\frac{q}{V_D} + \sum z_i \left(c_{D,i} - c_i\right) = 0 \qquad (1)$$

where q is the net charge of the HAs and FAs in mol kg^{-1}, VD is the Donnan volume or the specific volume of electrolyte in the gel phase in L kg^{-1}, z_i refers to the ionic charge of the ion i (including sign), $c_{D,i}$ is the molar concentration of the ion i in the gel phase, and c_i is the molar concentration of the ion i in the bulk solution. According to the Boltzmann distribution law, $c_{D,i}$ can be related to c_i by

$$c_{D,i} = c_i \exp\left(\frac{-z_i F \psi_D}{RT}\right) \qquad (2)$$

where F is the Faraday constant in C mol^{-1}, ψ_D is the Donnan potential in Volt, R is the gas constant in Joule mol^{-1} K^{-1}, and T is the absolute temperature in K. The exponential term is often called Boltzmann factor ($\chi_{D,i}$). The Donnan model requires that V_D is known over the complete range of solution conditions. According to Benedetti et al. (1996a), V_D decreases with increasing ionic strength following the empirical relationship

$$\log V_D = b\left(1 - \log I\right) - 1 \qquad (3)$$

where I is the ionic strength and b is an empirical parameter describing how V_D varies with ionic strength.

When a continuous distribution of binding sites is assumed, the fraction of all sites occupied by the species i (θ_i) is given by the following integral equation

$$\theta_i = \int_{\Delta \log Ki} \theta_{i,l} f\left(\log K_i\right) d\left(\log K_i\right) \qquad (4)$$

where $\theta_{i,l}$ is the local adsorption isotherm, which describes the binding of i to a group of identical sites, f(log K_i) is the distribution function of the affinity constant K_i, and Δ log K_i is the range of possible log K_i values. For a multicomponent system, the use of the competitive extended Hill equation, as the local isotherm, in combination with the Sips distribution function (quasi-Gaussian), leads to the basic NICA equation for binding of ions i in the competitive situation (Koopal et al., 1994, 2001; Benedetti et al., 1995, 1996a; Kinniburgh et al., 1996)

$$\theta_i = \frac{\left(\tilde{K}_i c_{D,i}\right)^{n_i}}{\sum_i \left(\tilde{K}_i c_{D,i}\right)^{n_i}} \times \frac{\left[\sum_i \left(\tilde{K}_i c_{D,i}\right)^{n_i}\right]^p}{1+\left[\sum_i \left(\tilde{K}_i c_{D,i}\right)^{n_i}\right]^p} \qquad (5)$$

where $\tilde{K}_i$ represents the median affinity constant for the component i, p is the width of the affinity distribution, which reflects the intrinsic chemical heterogeneity of HS, and n_i represents the stoichiometry of the binding reaction.

The bound amount of ion i, Q_i, is given by

$$Q_i = \theta_i n_i Q_{max} \qquad (6)$$

where Q_{max} is the overall site density, and the stoichiometry factor n_i reflects the number of ions i bound to each reference site. When the proton is used as reference ion the overall site density becomes that of the proton and the bound amount of ion i should be scaled by n_i/n_H rather than by n_i alone:

$$Q_i = \theta_i \left(\frac{n_i}{n_H}\right) Q_{max,H} \qquad (7)$$

Proton affinity distributions derived from acid-base titrations of HAs and FAs typically exhibit two broad peaks, indicating that there are two major groups of binding sites

which can be indicated as low and high affinity sites (De Wit et al., 1993; Milne et al., 1995; Fiol et al., 1999; Plaza et al., 2005c). The NICA model can be extended to reflect a bimodal distribution of proton affinities by

$$Q_i = \theta_{i,1}\left(\frac{n_{i,1}}{n_{H,1}}\right)Q_{max,1} + \theta_{i,2}\left(\frac{n_{i,2}}{n_{H,2}}\right)Q_{max,2} \qquad (8)$$

where the suffixes 1 and 2 denote two types of binding sites. Thus, for proton binding by HS in the absence of other specific cation binding, the amount of protons bound, Q_H, is given by

$$Q_H = Q_{max,1}\frac{\left(\tilde{K}_{H,1}c_{D,H}\right)^{m_1}}{1+\left(K_{H,1}c_{D,H}\right)^{m_1}} + Q_{max,2}\frac{\left(\tilde{K}_{H,2}c_{D,H}\right)^{m_2}}{1+\left(K_{H,2}c_{D,H}\right)^{m_2}} \qquad (9)$$

where m_1 and m_2 define the widths of the proton affinity distributions ($m_j = n_{H,j} \times p_j$; j = 1, 2) (Kinniburgh et al., 1996). The total amount of protons bound, $Q_{H,t}$, equals the sum of protons specifically bound and the amount of protons bound electrostatically in the Donnan phase

$$Q_{H,t} = Q_H + V_D\left(c_{D,H} - c_H\right) \qquad (10)$$

VII.2.5. Parameter estimation

The ionic strength and the proton concentration were calculated for each titration point by an iterative procedure from the concentration of the background electrolyte and pH. Activity coefficients were derived from the calculated ionic strength using the Davies equation (Davies, 1962). The relative net charge on HAs and FAs at a given proton concentration and ionic strength, which equals the relative amount of protons bound to HAs and FAs (ΔQ_H), was calculated by subtracting theoretical blank titrations from HA and FA titrations, i.e., by subtracting, for each data point, the amount of titrant required to increase

the pH of an equivalent volume of background electrolyte solution. In order to obtain the absolute amount of protons bound, the value of the initial amount of proton binding (Q_{H0}) was added to ΔQH for each point on the titration curve

$$Q_{H,t} = \Delta Q_H + Q_{H0} \qquad (11)$$

Once the ΔQ_H, the free proton concentration and ionic strength were calculated for each titration point, the optimum set of NICA-Donnan model parameters (i.e., b, $Q_{max,1}$, $Q_{max,2}$, $\widetilde{K}_{H,1}$, $\widetilde{K}_{H,2}$, m_1, and m_2) for each HA and FA sample was obtained by iteratively varying the adjustable parameter values (including Q_{H0}) until the sum of the squares of the differences between observed and fitted values of ΔQ_H was minimized. Titration data at pH < 3.5 or pH > 10.5 were considered unreliable, and were not used for calculations. Full, unconstrained optimization was achieved using a hybrid algorithm based on the Marquadt-Levenberg approach. The computer program FIT 2.5 was used for calculations (Kinniburgh, 1993).

VII.3. RESULTS AND DISCUSSION

VII.3.1. Proton binding isotherms

The negative charge on HAs and FAs isolated from CS, TS, and unamended (**Figure VII.1**) and amended soils (**Figure VII.2**) increases with increasing pH and ionic strength in the bulk solution as a result of proton dissociation and a combination of Coulombic effects and site heterogeneity (Tipping, 2002). The titration curves for each ionic strength are nearly parallel until neutral pH and tend to converge slightly above pH 7. These results indicate that the effect of ionic strength on the charge developed by HAs and FAs is slightly greater at low pH than at high pH. Similar trends are described in previous studies on HS of different origins (Bartschat et al., 1992; Milne et al., 1995; Christensen et al., 1998; et al., 2005ab, 2006b). Also in agreement with previous findings (Milne et al., 2001; Ritchie and Perdue,

2003; Plaza et al., 2006b), the HAs exhibit charge densities smaller than the corresponding FAs across the entire range of proton concentration and ionic strength.

At any given ionic strength, the charge densities of CS-HA are slightly smaller than the values of TS-HA until about pH 6 and slightly larger above pH 6, whereas the charge of CS-FA is slightly larger than that of TS-HA across the entire range of proton concentration. In general, the charge densities of HAs and FAs from CS and TS are slightly smaller or smaller than the values of soil HAs and FAs, which tend to decrease as an effect of the amendment with CS and especially TS.

VII.3.2. NICA-Donnan parameters

The values of the NICA-Donnan model parameters for a bimodal distribution of proton binding sites that best fit the experimental titration datasets of each HA and FA sample are listed in Table 1. The large values of the determination coefficient (r^2), the small residual mean square errors (RMSE) and the best-fit lines generated from these parameters (**Figures VII.1** and **VII.2**) show that the bimodal NICA-Donnan model provides an accurate description of the experimental datasets for proton binding to the HAs and FAs examined. The parameter b, the contents of binding sites with low and high proton affinities ($Q_{max,1}$ and $Q_{max,2,}$ respectively), the median values of affinity distributions for proton binding to low and high affinity sites ($\widetilde{K}_{H,1}$ and $\widetilde{K}_{H,2}$, respectively), and the width of proton affinity distributions of low and high affinity binding sites (m_1 and m_2, respectively) of HAs and FAs estimated by the NICA-Donnan model are discussed comparatively in the following text. In agreement with the conventional interpretation of acid-base properties of HS (Perdue, 1985; Stevenson, 1994; Masini et al., 1998; Koopal et al., 2005), the two major types of acidic functional groups with low and high proton affinities are assumed to be, respectively, carboxylic- and phenolic-type groups. **Figure VII.3** shows the proton affinity distributions associated with the NICA-Donnan descriptions, which were calculated from the first derivative of the curve model-derived values of charge versus proton concentration in the Donnan phase by applying the condensation approximation method (Nederlof et al., 1992).

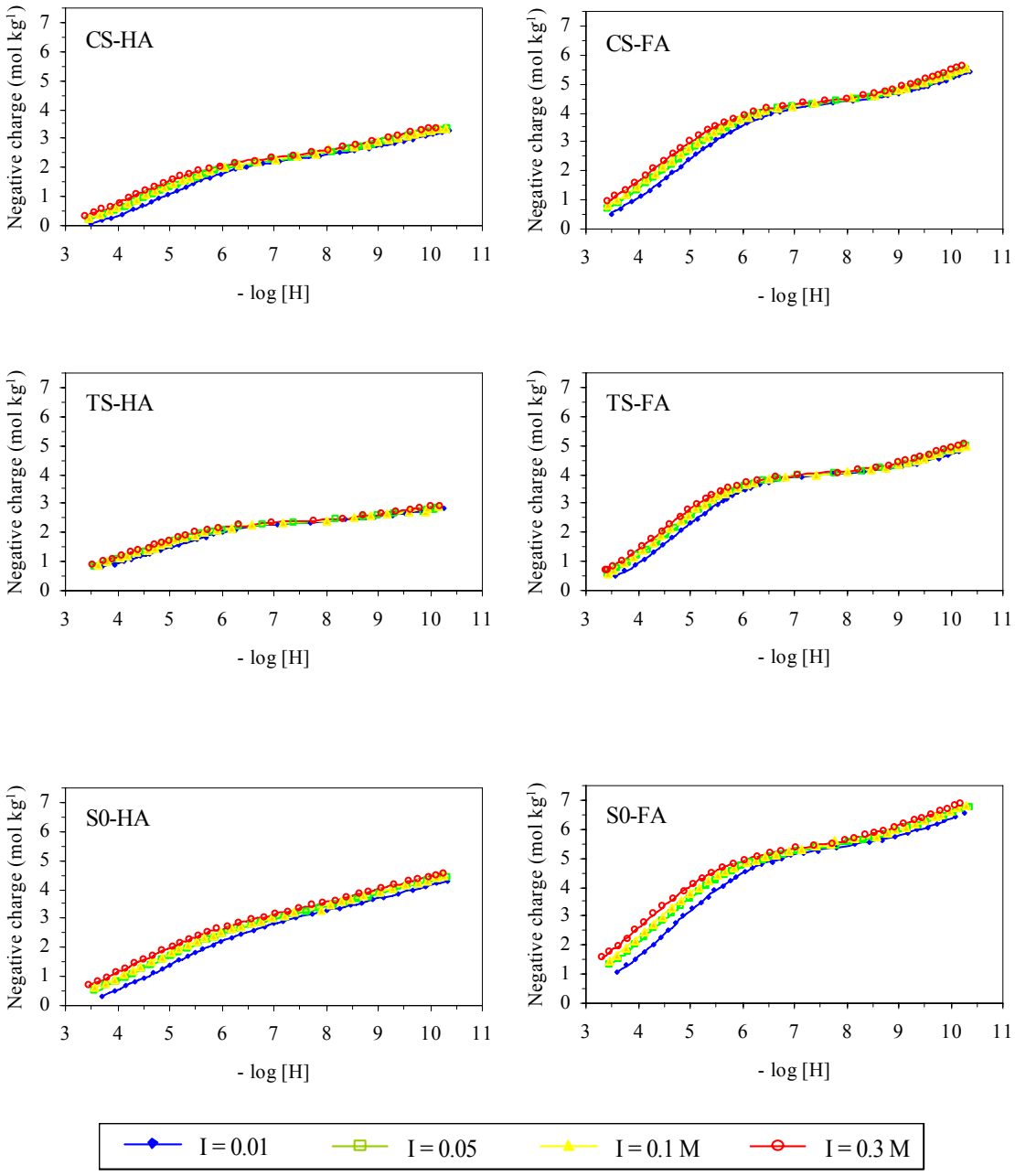

Figure VII.1. Negative charge of humic acids (HAs) and fulvic acids (FAs) isolated from composted sewage sludge (CS), thermally-dried sewage sludge (TS) and the corresponding unamended soil (S0) as a function of proton concentration and ionic strength (I). Symbols represent experimental data obtained from potentiometric titrations (only half of the data is shown for clarity); continuous lines represent fits with the NICA-Donnan model.

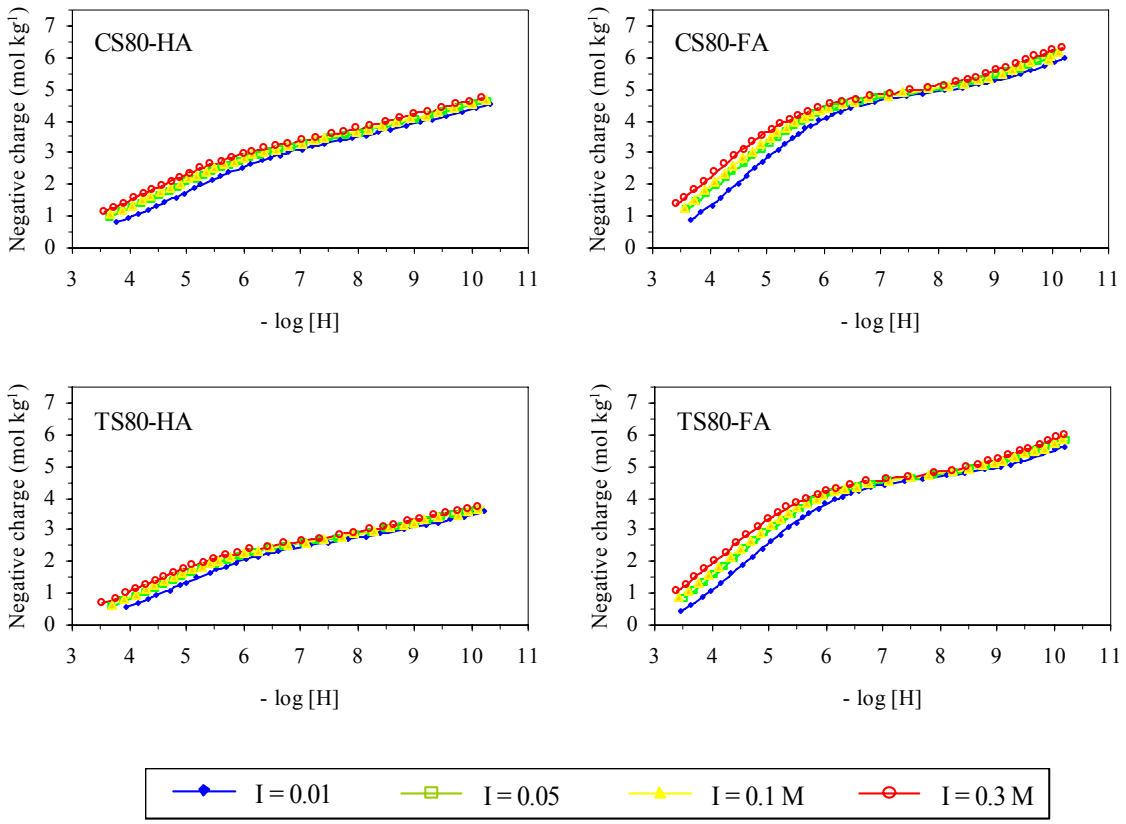

Figure VII.2. Negative charge of humic acids (HAs) and fulvic acids (FAs) isolated from soils amended with either CS or TS at a rate of 80 t ha^{-1} yr^{-1} for 3 years (CS80 and TS80, respectively), and the corresponding unamended soil (S0) as a function of proton concentration and ionic strength (I). Symbols represent experimental data obtained from potentiometric titrations (only half of the data is shown for clarity); continuous lines represent fits with the NICA-Donnan model.

VII.3.2.1. Parameter b

The b values of HAs from CS, TS, unamended and amended soils are smaller than those of the corresponding FAs, which is consistent with results recently reported by Milne et al. (2001) for generic HAs and FAs (**Table VII.1**). Further, the b values of CS-HA and CS-FA are smaller than the b values of TS-HA and TS-FA, which in turn are much larger than the corresponding values of S0-HA and S0-FA. Amendment with CS and especially with TS induces a slight increase of the b values of soil HAs and FAs. According to Christl

and Kretzschmar (2001), the parameter b is correlated negatively with particle size and molecular weight. Larger b values in CS-HA and CS-FA and especially in TS-HA and TS-FA, as compared to soil HAs and FAs, might thus suggest a greater content of small molecular weight particles in the formers. This result agrees with previously-reported gel-chromatographic analysis of molecular weight distribution of HS isolated from similar systems (Trubetskaya et al., 2001).

Table VII.1: Fitting parameters [a] of the NICA-Donnan model for proton binding to humic acids (HAs) and fulvic acids (FAs) isolated from composted sewage sludge (CS), thermally-dried sewage sludge (TS), soils amended with either CS or TS at a rate of 80 t ha^{-1} yr^{-1} for 3 years (CS80 and TS80, respectively), and the corresponding unamended soil (S0)

HA and FA samples	r^2	RMSE	b	$Q_{max,1}$	$\log \widetilde{K}_{H,1}$	m_1	$Q_{max,2}$	$\log \widetilde{K}_{H,2}$	m_2	$Q_{max,1} + Q_{max,2}$
CS-HA	0.99991	0.0090	0.61	2.67	3.43	0.66	1.66	8.05	0.43	4.33
TS-HA	0.99951	0.0137	0.70	1.92	3.91	0.70	0.76	8.53	0.59	2.69
S0-HA	0.99991	0.0107	0.53	3.57	3.07	0.50	2.32	7.72	0.35	5.88
CS80-HA	0.99965	0.0196	0.55	3.38	3.15	0.52	2.08	7.75	0.38	5.46
TS80-HA	0.99986	0.0105	0.60	3.03	3.21	0.56	1.70	7.99	0.42	4.74
CS-FA	0.99985	0.0169	0.69	4.91	3.31	0.63	1.93	8.53	0.59	6.83
TS-FA	0.99994	0.0093	0.74	4.57	3.45	0.72	1.52	8.63	0.67	6.10
S0-FA	0.99986	0.0186	0.58	6.09	2.68	0.57	2.42	8.17	0.50	8.51
CS80-FA	0.99986	0.0168	0.59	5.79	2.73	0.58	2.27	8.19	0.54	8.06
TS80-FA	0.99992	0.0129	0.62	5.69	2.88	0.58	2.11	8.37	0.58	7.80

[a] r^2, coefficient of determination; RMSE, root mean square error after optimization; b, empirical parameter describing how the Donnan volume varies with ionic strength; $Q_{max,1}$, carboxylic-type group content (mol kg^{-1} on moisture- and ash-free basis); $\widetilde{K}_{H,1}$, median value of affinity distribution for proton binding by carboxylic-type groups; m_1, width of proton-affinity distribution of carboxylic-type groups; $Q_{max,2}$, phenolic-type group content (mol kg^{-1} on moisture- and ash-free basis); $\widetilde{K}_{H,2}$, median value of affinity distribution for proton binding by phenolic-type groups; m_2, width of proton-affinity distribution of phenolic-type groups.

VII.3.2.2. Site density

The overall site density (or total acidity) and carboxylic-type group contents ($Q_{max,1}$ + $Q_{max,2}$ and $Q_{max,1}$, respectively, in **Table VII.1**) of HAs are much smaller than those of FAs, whereas the phenolic-type group contents ($Q_{max,2}$ in **Table VII.1**) are smaller or slightly smaller. Carboxyl groups constitute 61% to 71% of the total acidity for HAs and 72% to 75% for FAs. These results agree with data on a large number of samples in indicating that most of the acidity of HAs, and especially of FAs, is attributable to carboxyl groups (Milne et al., 2001; Ritchie and Perdue, 2003).

The total acidity and carboxylic- and phenolic-type group contents of CS-HA are larger than those of S0-HA. With respect to S0-HA, CS-HA and TS-HA have, respectively, smaller and much smaller acidic functional group contents. Further, the acidic functional group content of CS-FA is slightly larger than that of TS-FA, and both are smaller than that of S0-FA. These results may be attributed to the well-aerated conditions existing during the composting process and in soil, which favor oxidative polymerization and other oxidation reactions leading to the formation of acidic oxygenated functional groups. In comparison with S0-HA and S0-FA, the HA and FA fractions from CS- and TS-amended soils have slightly smaller carboxylic- and phenolic-type group contents. These effects are more evident for TS-amended soil HA and FA than for CS-amended soil HA and FA.

Table VII.2 shows the acidic functional group contents of HAs and FAs isolated from CS, TS, and unamended and amended soils measured by direct titrations using a pH-based method (Ritchie and Perdue, 2003). Briefly, the carboxyl group content was estimated as the value of charge at pH 8 and ionic strength 0.1 M, phenolic hydroxyl group content was estimated as two times the change in charge between pH 8 and pH 10, and total acidity was calculated by addition. Except for the carboxyl group contents of TS-HA and CS80-HA, the values of carboxyl and phenolic OH group contents obtained by the direct titration method are slightly smaller than the corresponding values obtained by using the NICA-Donnan model. However, the comparison of both sets of values reveals consistent trends which lead to the same general conclusions.

VII.3.2.3. Proton binding affinities

Table VII.2: Acidic functional group contents [a] of humic acids (HAs) and fulvic acids (FAs) isolated from composted sewage sludge (CS), thermally-dried sewage sludge (TS), soils amended with either CS or TS at a rate of 80 t ha^{-1} yr^{-1} for 3 years (CS80 and TS80, respectively), and the corresponding unamended soil (S0)

HA and FA samples	Total acidity	COOH	Phenolic OH
		(mol kg^{-1}) [b]	
CS-HA	3.94	2.58	1.36
TS-HA	3.05	2.40	0.65
S0-HA	5.23	3.30	1.94
CS80-HA	5.16	3.50	1.66
TS80-HA	4.12	2.87	1.26
CS-FA	6.17	4.58	1.58
TS-FA	5.56	4.05	1.51
S0-FA	7.50	5.62	1.87
CS80-FA	6.74	5.04	1.70
TS80-FA	6.41	4.77	1.64

[a] Measured by direct titrations using the pH-based method

[b] On moisture- and ash-free basis.

The median values of affinity distributions for proton binding by carboxylic- and phenolic-type groups (log $\tilde{K}_{H,1}$ and log $\tilde{K}_{H,2}$, respectively) derived for the HAs and FAs examined correspond very well to values commonly reported for HAs and FAs (Benedetti et al., 1996ab; Kinniburgh et al., 1996; Christensen et al., 1998; Pinheiro et al., 1999; Christl and Kretzschmar, 2001; Milne et al., 2001; Plaza et al., 2005ab, 2006b), and closely resemble proton affinity constants of mono- and polycarboxylic acids and phenols (Christensen et al., 1998; Christl and Kretzschmar, 2001) (**Table VII.1**). This result strongly supports the statement that proton binding to HAs and FAs is mainly determined by carboxyl and phenolic OH groups. In agreement with results reported by Milne et al. (2001) for

generic HAs and FAs, the median values of affinity distributions for proton binding to carboxylic-type groups (log $\widetilde{K}_{H,1}$) are larger for the HAs than for the corresponding FAs (**Table VII.1**), whereas the median values of affinity distributions for proton binding to phenolic-type groups (log $\widetilde{K}_{H,2}$) are smaller for the HAs than for the corresponding FAs.

The log $\widetilde{K}_{H,1}$ and log $\widetilde{K}_{H,2}$ of TS-HA are larger than the log $\widetilde{K}_{H,1}$ and log $\widetilde{K}_{H,2}$ of CS-HA, which are in turn larger than those of S0-HA. Further, the log $\widetilde{K}_{H,1}$ and log $\widetilde{K}_{H,2}$ of CS-FA are slightly smaller than those of TS-FA, and both are larger than the corresponding values of S0-FA. The amended soil HAs and FAs feature slightly larger log $\widetilde{K}_{H,1}$ and log $\widetilde{K}_{H,2}$ than S0-HA and S0-FA, respectively. These results indicate that the carboxylic- and phenolic-type groups of the HA and FA fractions of CS, and especially TS, are less acidic than those of unamended soil HA and FA, and that SS application causes a slight decrease of acidity of soil HAs and FAs which is slightly more evident in the HA and FA from TS-amended soil.

VII.3.2.4. Width of the affinity distributions

In agreement with previous findings (Benedetti et al., 1996ab; Kinniburgh et al., 1996; Christl and Kretzschmar, 2001; Pinheiro et al., 1999; Plaza et al., 2005ab, 2006b), the values of m_1 of all HAs and FAs examined are larger than the corresponding values of m_2, with the only exception of TS80-FA which shows similar values of m_1 and m_2 (**Table VII.1**). According to Koopal et al. (2005), these results indicate that the low affinity proton binding sites (carboxylic-type groups) exhibit a smaller apparent heterogeneity than the high affinity sites (phenolic-type groups). Further, with the exception of CS-HA, which features a slightly larger m_1 value than its FA counterpart, the m_1 and m_2 values of the HAs examined are smaller than the corresponding values of FAs, which indicates a greater heterogeneity of acidic functional groups in the former samples.

The m_1 and m_2 values of CS-HA are, respectively, smaller and much smaller than the corresponding values of TS-HA. Further, as compared to the corresponding values of S0-HA, the values of m1 and m_2 of CS-HA are, respectively, much larger and larger, whereas the m_1 and m_2 values of TS-HA are much larger. These results indicate that the HA fractions of the SS studied, especially of TS, feature a greater homogeneity in carboxylic- and phenolic-type groups than soil HA. Amendment with CS, and especially with TS, induces an increase of the m1 and m_2 values of amended soil HAs, i.e., a decrease of the heterogeneity of acidic functional groups.

The values of m_1 and m_2 of TS-FA are larger than the corresponding values of CS-FA, which are in turn larger than those of S0-HA. These results indicate that, similar to the HA fractions, the FA fractions of CS, and especially TS, feature a greater homogeneity in carboxylic- and phenolic-type groups than soil FA. Similar to the effect on the HA fractions, the application of both SS induces a decrease of the m_1 value (i.e., an increase of the heterogeneity of the carboxylic-type groups) of amended soil FAs, which is more marked in the case of TS, and does not affect substantially the heterogeneity of the m_2 value (i.e, the heterogeneity of phenolic-type groups).

VII.3.2.5. Proton affinity distributions

The distributions of proton binding sites of CS-HA and S0-HA associated with the NICA-Donnan parameters discussed above differ slightly from each other and markedly from that of TS-HA. Similarly, the distribution of proton binding sites of CS-FA resembles the distribution of S0-FA more than that of TS-FA (**Fig VII.3**). Application of CS, and especially TS, modifies the proton affinity distributions of native soil HA and FA. In general these results suggest the possible partial association of HA and FA fractions of CS and TS to native soil HA and FA. Apparently, the composting process produces HA and FA fractions with acid-base properties that more closely resemble those typical of native soil HAs and FAs, and therefore induces only minor modifications of the properties of HA and FA fractions in CS-amended soil.

VII.4. CONCLUSIONS

The NICA-Donnan model provides an accurate description of the acid-base properties of the HAs and FAs form CS, TS, and unamended and amended soils with chemically reasonable parameters. When compared to unamended soil HA and FA, HAs and FAs from CS, and especially TS, have smaller acidic functional group contents and larger proton binding affinities and smaller heterogeneity of carboxylic and phenolic-type groups. Amendment with CS or TS causes a decrease of acidic functional group contents and a slight increase of proton binding affinities of carboxylic- and phenolic-type groups of soil HAs and FAs. These effects are more evident in the HA and FA fractions from CS-amended soil than in those from TS-amended soil. In conclusion, composting, as compared to thermal drying, produces HA and FA fractions with acid-base properties that more resemble those typical of native soil HAs and FAs and, therefore, induce fewer modifications in amended soil HAs and FAs.

VIII. BIOCHEMICAL PROPERTIES AND BARLEY YIELD IN A SEMIARID MEDITERRANEAN SOIL AMENDED WITH TWO KINDS OF SEWAGE SLUDGE

VIII. BIOCHEMICAL PROPERTIES AND BARLEY YIELD IN A SEMIARID MEDITERRANEAN SOIL AMENDED WITH TWO KINDS OF SEWAGE SLUDGE

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INDEX

ABSTRACT

Sewage sludges from wastewater treatment plants are typically rich in organic matter contents and in macro- and micronutrients, especially N and P. As a result, recycling of sewage sludge as soil amendments represents a promising agricultural practice, especially in Mediterranean zones which soils are naturally poor in organic mater. However, this organic waste must be treated to avoid possible deleterious effects over the soil or the plants. For this aim, a composted and a thermally dried sewage sludges have been applied to a cropped soil over a three years period, with different frequencies (yearly or single) and at to different rates (20 t ha^{-1} and 80 t ha^{-1}), to study their cumulative and residual effects on the yield of barley grown and on chemical, biological and biochemical soil properties. In the cumulative experiment high doses of sewage sludge cause an increase of the total organic C contents but a decrease in crop yield, as well as in enzymes activities and microbial biomass C contents. In contrast, the low cumulative doses of both sewage sludges, but especially of the composted one, show better results for the parameters studied. However, the best results for barley yield were obtained for the soils amended once with sewage sludge, particularly those amended with the composted sewage sludge. These results may be attributed to a possible deleterious effect over soil properties derived from the use of excessive doses. Besides, results stand out the benefits derived from the application to the soil of the composted sewage sludge, which presents an organic matter more mature and stable than the thermally-dried sewage sludge

Keywords: Composting; Thermal-drying; Sewage sludge; Barley yield; Soil enzymes; Microbial biomass

VIII.1. INTRODUCTION

Mediterranean soils are often subjected to severe degradation processes accompanied by a decline of soil organic matter contents, which affects negatively soil fertility (Smith et al., 1993) and increases the risks of erosion and desertification, especially in agricultural ecosystems (Plaza et al., 2004; Senesi et al., 2006). One common way to reverse this soil quality degradation is through the use of organic amendments, which allow restoring soil organic matter content and its physical, chemical, and biological functions (Stevenson, 1994, Senesi et al., 1996). Because of traditional organic soil amendments, such as farmyard manure and peat, are locally scarce (Saviozzi et al., 1999), a wide variety of organic wastes of various sources and generated in great amounts are currently used. In addition, recycling of these materials into the soil system reduces the waste stream disposed in landfills or incineration plants (Eriksen et al., 1999; Trinsoutrot et al., 2000; Tejada and González, 2003; Plaza et al., 2006b; Fernandez et al., 2007a).

Sewage sludges (SS) are organic C-rich materials produced during wastewater treatments and represent a source of organic matter, N, P, and other nutrients, which, if properly managed, can be used advantageously to enhance organic fertility in intensively-cropped degraded soils (Albiach et al., 2001; García-Gil et al., 2004). Because of the use of fresh organic wastes can affect negatively soil properties and plant growth (Senesi et al., 1996; Plaza et al., 2003), raw SS should be subjected to appropriate treatment before land application. In many cases, SS are composted with the aim of obtaining their chemical stabilization, biological maturation, and sanitization, and thus a useful soil amendment with low risks to the environment (Senesi, 1989). However, in the last years thermal drying technologies, which transforms SS into a dry and pathogen-free granular product easy to be transported and handled, is becoming more and more important in wastewater treatment plants (Gavalda et al., 2005; Fernández et al., 2007b).

Wide evidence exists in the literature that the addition of good quality composts may enhance soil physical, physicochemical and chemical properties, as well as affect positively microbiological and biochemical factors, such as soil microbial biomass and enzyme

activities, which are potentially involved in biogeochemical cycles and can exert a great influence on plant productivity parameters. However, little is known about the specific effects of thermally-dried materials, especially about their influence on soil biota and their long-term effects on crop yields.

The objective of this work is to investigate the cumulative and residual long-term effects of amendment with composted (CS) and thermally-dried sewage sludges (TS) at different rates on barley yield and on some chemical, biological, and biochemical soil properties in a semiarid Mediterranean agroecosystem.

VIII.2. MATERIALS AND METHODS

VIII.2.1. Sewage sludges

The CS sample was collected from a 3-month windrow-composted mixture of three SS originated from three municipal wastewater treatment plants in Madrid (Spain) metropolitan area. The composition of the CS sample (± standard errors of three laboratory replicates) was the following: pH, 7.1 ± 0.1; electrical conductivity (EC), 3.90 ± 0.01 dS m^{-1}; total organic C (TOC), 181.0 ± 0.2 g kg^{-1}; total N, 23.9 ± 0.1g kg^{-1}; total P, 13.90 ± 0.02 g kg^{-1}; and total K, 5.02 ± 0.07 g kg^{-1}. The TS sample consisted of a SS dried with hot air at temperatures between 380ºC and 450ºC in the wastewater treatment plant SUR in Madrid metropolitan area. The main chemical properties of the TS sample were the following: pH, 7.0 ± 0.1; EC, 1.50 ± 0.02 dS m^{-1}; TOC, 296.0 ± 0.2 g kg^{-1}; total N, 35.6 ± 0.1g kg^{-1}; total P, 13.43 ± 0.02 g kg^{-1}; and total K, 4.29 ± 0.05 g kg^{-1}. The chemical composition of both CS and TS generally fall within the ranges commonly reported in the literature for these kinds of SS (Albiach et al., 2001, Tarrason et al, 2007; Domene et al., 2007).

VIII.2.2. Soil and field experiment

The field experiment was conducted in the experimental farm "La Higueruela" located in Toledo (Spain) on a sandy loam soil (sand, 580 g kg^{-1}; silt, 240 g kg^{-1}; clay, 180 g kg^{-1}) classified as a Typic Haploxeralf (Soil Survey Staff, 2003). The site is characterized by a continental semiarid climate with an average annual rainfall of about 487 mm and an average annual temperature of 14 ºC.

The experimental design was performed in four randomised blocks of nonirrigated soil plots (20 × 3 m^2) cropped with barley (*Hordeum vulgare* L.). Soil plots were either unamended (S0) or amended with CS and TS at rates of 20 and 80 t ha^{-1} y^{-1} on a dry weight basis (CS20 and CS80, and TS20 and TS80, respectively). Both CS and TS were applied in mid-September, prior to barley planting in mid-October, and immediately incorporated into soil at a depth of 0-15 cm. After the first year of CS and TS application (2002), each plot was divided into two halves (10 × 3 m^2). In one half of the plots, the amendments with CS and TS were again applied for two more years (until 2004) in order to study the cumulative effects (C). In the other half of the plots, no application was made with the aim of evaluating the residual effects of the first amendments (R). Soil plots fertilized for 3 years with 400 kg ha^{-1} y^{-1} of NPK 15-15-15 were also included in the experimental design.

At the third barley harvest season (late June 2005), the entire above-ground barley biomass covering 1 m^2 of soil surface was collected from each plot. Soon after barley harvest, surface soil samples were collected randomly from the arable layer (Ap horizon, 0-20 cm depth) from each plot. Each soil sample consisted of a mixture of 10 soil cores of 3-cm diameter each. Soil samples for biological and biochemical analyses were stored at 4ºC at field moisture.

VIII.2.3. Barley yield analysis

The entire barley plant samples collected were weighed, and then the grain was separated from the straw and also weighed. Moisture contents of the grain and straw were determined by heating for 24 h at 105 °C. Total wheat plant and grain yields were calculated on a moisture-free basis, whereas straw yield was calculated as the difference between total

and grain yields. The harvest index was calculated as the percentage amount of grain in the total above-ground biomass. The number of spikes per square meter was determined by dividing the number of spikes contained in all plants in each plot by the plot area. The average kernel weight was calculated as the average of the weights of 1000 kernels taken randomly. The number of kernels per square meter was determined by dividing the grain yield by the average kernel weight.

VIII.2.4. Soil chemical analysis

Prior to analysis soil samples were air-dried and passed through a 2 mm sieve. The principal properties of soil samples were determined by standard methods (Sparks et al., 1996). In particular, the pH was measured on mixtures of soil:water = 1:2.5 and sludge:water = 1:5; the EC was measured on a 1:5 sample:water extract; and the TOC content was determined by dichromate oxidation of the sample and subsequent titration with ferrous ammonium sulphate. The total N content was obtained by the Kjeldahl method and spectrophtometric determination by a Bran-Lubbe Technicon autoanalyser AAII (Buffalo Grove, IL). Available P content was determined according to the Olsen method; and available K content was measured in 1 M $BaCl_2$ soil extracts using a ratio of soil to extractant of 1:10.

VIII.2.5. Soil biological and biochemical analyses

Biomass C was determined by fumigation of the sample with ethanol-free $CHCl_3$ and extraction with 0.5 M K_2SO_4, according to Vance et al. (1987). Prior to analysis, samples were incubated for 12 h at 25ºC. Dehydrogenase activity was determined by the reduction of 2-p-iodo-nitrophenyl-phenyltetrazolium chloride (INT) to iodo-nitrophenyl formazan (INTF) using the method of Skujins (1976) as modified by García et al. (1993). Catalase activity was determined by measuring the O_2 consumed by $KMnO_4$ after addition of H_2O_2 to the samples (Rodríguez-Kábana and Truelove, 1982). Urease activity was determined by measuring the NH_4^+ released using urea as substrate (Nannipieri et al., 1980).

VIII.2.5. Statistical analysis

Analytical data were subjected to an analysis of variance using the least significant difference test when F-test was significant at a 0.05 probability level. Correlation analyses were made with all the agronomical, chemical, biological and biochemical data. The Statgraphics Plus 2.0. software was used for calculations.

VIII.3. RESULTS AND DISCUSSION

VIII.3.1. Barley yield

The barley yield parameters of the unamended and the variously amended soils are shown in **Tables VIII.1** and **VIII.2**. The application of CS and TS to soil for three consecutive years determines significant increases of straw and total dry matter yields with respect to the unamended and mineral-fertilized soils (**Table VIII.1**). However, only the soil amended with 20 t ha^{-1} y^{-1} of TS has a significantly higher grain yield with respect to the unamended soil (**Table VIII.1**). In general, the harvest index and kernel weight are lower in sludge-amended soils than in unamended and mineral-fertilized soils, whereas the contrary is true for spike and kernel numbers per square meter (**Table VIII.1**).

In general, except for total and straw yields, soils amended once with TS, and especially CS, give better barley yield parameters than the corresponding soils amended for three consecutive years (**Table VIII.2**). In particular, the single application of CS and TS to soil increases grain, straw and dry matter yields, and spike and kernel number per square meter, whereas does not affect negatively the harvest index and kernel weight.

The excessive growth of straw in relation with grain and the resultant low harvest indexes measured in soils amended with TS and CS for three consecutive years could be feasibly ascribed to the great amount of N added with both kinds of sludges. When water supply is adequate, very high levels of N can over-stimulate the growth, and the plant

accumulates more biomass. In this period, water-soluble carbohydrates (stem sugars) are locked up by the plant in this structures rather than leaving them in storage to be used later to fill the grains at the time of flowering. As a result, when the season progresses and water becomes inadequate to support that increased biomass, the shortage of these stem sugars cause the yield reduction and the pinched grain obtained (Dilz, 1981). Indeed, a significantly negative relation ($P < 0.01$) exits between N contents and harvest index in the cumulative experiment (**Tables VIII.5 and VIII.6**).

Table VIII.1: Barley yield parameters of control soil (S0), mineral-fertilized soil, and soils amended with 20 and 80 t ha^{-1} of composted sewage sludge (CS20-C and CS80-C, respectively) and thermally-dried sewage sludge (TS20-C and TS80-C, respectively) in the cumulative treatments.

	Dry matter yield			Harvest index	Spikes	Kernels	
	Grain	Straw	Total				Average weight
	(t ha^{-1})	(t ha^{-1})	(t ha^{-1})	(%)	(Units m^{-2})	(Units m^{-2})	(mg)
S0	1.2ab	1.3a	2.5a	47.7c	300.7a	3130.1ab	39.4d
MF	1.6bc	1.8a	3.3b	46.3c	329.4a	4095.5bc	38.1cd
CS20-C	1.9bc	3.3bc	5.2d	35.8bc	458.3ab	5102.7c	36.3bc
CS80-C	0.7a	3.4c	4.1c	16.4a	428.3ab	2116.7a	31.8a
TS20-C	2.0c	4.2d	6.2e	31.4b	510.0b	5143.9c	39.0d
TS80-C	1.5bc	2.7b	4.2c	35.5bc	396.6ab	4163.4bc	35.5b

Values of the same column followed by the same letter are not significantly different according to LSD test (P=0.05).

VIII.3.2. Soil chemical properties

In comparison with unamended and mineral fertilized soils, soils amended yearly with TS, and especially CS, over a 3-year period, have higher values of pH and EC, and larger contents of TOC, total N, and available P and K. These effects are more evident with increasing the amendment rate. In the residual experiment, the chemical properties of the unamended soil and the SS-amended soils differ very slightly from each other. The only

exception is TOC content, which is higher for the soil amended once with 80 t ha^{-1} y^{-1} of TS (TS80-R) than for the unamended soil.

Table VIII.2: Barley yield parameters of control soil (S0), mineral-fertilized soil (MF), and soils amended with 20 or 80 t ha^{-1} of composted sewage sludge (CS20-R and CS80-R, respectively) or thermally-dried sewage sludge (TS20-R and TS80-R, respectively) in the residual treatments.

	Dry matter yield			Harvest index	Spikes	Kernels	
	Grain	Straw	Total				Average weight
	(t ha^{-1})	(t ha^{-1})	(t ha^{-1})	(%)	(Units m^{-2})	(Units m^{-2})	(mg)
S0	1.2a	1.3a	2.5a	47.7ab	300.7a	3130.1a	39.4ab
MF	1.6ab	1.8a	3.3b	46.3ab	329.4a	4095.5ab	38.1a
CS20-R	2.1abc	2.3ab	4.4c	48.4ab	539.3bc	5404.5bc	38.9a
CS80-R	2.8c	2.1a	4.8cd	57.3b	673.3c	6875.4c	40.3ab
TS20-R	2.0abc	3.3c	5.3de	38.1a	457.8ab	4841.5abc	41.8b
TS80-R	2.3bc	3.2bc	5.5e	42.1ab	501.1abc	5853.0bc	39.6ab

Values of the same column followed by the same letter are not significantly different according to LSD test (P=0.05).

The significant increase of pH, TOC, EC, total N and available P and K in cumulative-amended soils may be directly related to the continuous applications of CS and TS and to the high contents of organic matter, N, P, K and soluble salts in these sludges. In the residual experiments, the low differences found between the chemical properties of the unamended soil and soils amended once, may be attributed, mainly, to nutrient uptake by crops over the 3 years of experiment, as well as to other natural processes such as C and N mineralization and subsequent losses by CO_2 release, NO_3^- leaching, and NH_3 volatilization.

It is noteworthy that, despite of the larger TOC content of TS as compared to CS, the higher increase of TOC is measured in the cumulative CS-amended soils, and not in those amended with TS, which suggests the occurrence of less extended mineralization processes in the formers. In agreement with previous results (Fernández et al, 2007b), this effect may be attributed to the larger presence of easily biodegradable compounds in TS than in CS.

Table VIII.3: Main chemical properties (± standard errors) of control soil (S0), soil amended with mineral fertilizer (MF), and soils amended with 20 and 80 t ha^{-1} of composted sewage sludge (CS20 and CS80) or thermally-dried sewage sludge (TS20 and TS80) in the cumulative (-C) and residual (-R) treatments.

Property	S0	MF	CS20-C	CS80-C	TS20-C	TS80-C	CS20-R	CS80-R	T0S20-R	TS80-R
pH	5.7±0.1	5.7±0.1	6.5±0.1	6.5±0.1	6.0±0.2	6.2±0.1	5.8±0.2	6.1±0.2	5.4±0.0	5.9±0.2
E.C. (dS m^{-1})	0.05±0.01	0.04±0.01	0.07±0.02	0.29±0.01	0.06±0.00	0.12±0.02	0.03±0.01	0.05±0.02	0.03±0.01	0.04±0.01
Total Organic C (g kg^{-1})	7.2±0.1	5.8±0.1	17.0±0.8	31.0±0.5	15.2±0.8	20.3±0.5	6.2±0.8	7.3±0.7	7.9±0.7	12.3±0.7
Total N (g kg^{-1})	0.9±0.2	0.6±0.1	1.7±0.1	4.5±0.3	1.5±0.2	3.0±0.4	0.6±0.0	0.8±0.2	0.6±0.1	1.0±0.2
Available P (g kg^{-1})	0.09±0.01	0.10±0.03	0.18±0.04	0.58±0.02	0.07±0.00	0.16±0.03	0.04±0.01	0.07±0.02	0.03±0.01	0.07±0.01
Available K (g kg^{-1})	0.20±0.01	0.20±0.01	0.21±0.04	0.31±0.03	0.18±0.01	0.25±0.02	0.18±0.02	0.16±0.02	0.19±0.04	0.18±0.02

Unlike CS, TS organic matter has not been stabilized during the thermal-drying treatment, and its addition to soil may lead to an initial growth of the soil microbial population, which would strongly mineralize the organic C sources incorporated with TS to soil. On the contrary, when the CS is added to soil, mineralization processes are slower as result of the higher organic matter stability achieved during the maturation phase of composting, which involves a lowest loss of TOC and a greatest degree of C conservation (Bernal et al., 1998; Fernández et al., 2007a).

VIII.3.3. Soil microbial biomass and enzyme activities

In general, the highest microbial biomass C contents are measured in the repeatedly-amended soils, particularly in that amended with the lowest rate of CS (**Table VIII.4**). The ratio of microbial biomass C to TOC is also higher for soils amended with the lowest rate of SS, especially for that amended with CS, but in this case, the residual and not the cumulative experiments, feature the highest values (**Table VIII.4**).

The microbial biomass C has been considered as a more sensitive indicator of changing soil conditions than is the TOC content (García-Gil et al., 2000). The increase of microbial biomass in the cumulative and residual experiments, after the amendment with SS, can be attributed to the incorporation to the soil of easily-degradable organic compounds contained in the sludges. These substrate-C stimulate the growth of the autochthonous soil microbiota due to the greater availability of energy sources, as has been confirmed by prior analysis (Antolín et al. 2005; Pascual et al., 2007).

The negative effect of increasing the doses of sludge measured on the biomass C content is confirmed by the results obtained for the microbial biomass C/TOC ratio, whose highest values correspond to the soils amended once with only 20 t ha^{-1}. These effects could be attributed to several factors. Firstly, it has been reported that large fertilizer additions lead to reductions in microbial activity, due to, at least partially, changes in pH (Insam et al., 1995), although in this case great variations on the pH of the soils amended were not observed. The negative effect of high EC has also been described as a deleterious effect over

microbial population (Simek et al., 1999; Thirukkumaran and Parkinson, 2000). In the present study, this factor could be more relevant, although no significant correlation has been found between microbial biomass C content and EC (**Table VIII.5** and **Table VIII.6**). Furthermore, according to a number of studies (Flieβbach et al., 1994; Filip and Bielek, 2002), a reduction of microbial biomass C could be a result of metal pollution. The microbial biomass C/TOC ratio has been proposed by Brookes (1995) as a useful measure of soil pollution by heavy metals. Although a low ratio could be attributed to the heavy metals added with the amendment at the high rate, it could be also attributed to a high condensation and humification degree of soil organic matter resistant to microbial attack (Tate et al, 1987; García-Gil et al., 2000).

Table VIII.4: Soil microbial biomass C content (mg kg^{-1}) and microbial biomass C/Total organic C ratio of control soil (S0), mineral-fertilized soil, and soils amended with 20 and 80 t ha^{-1} of composted sewage sludge (CS20 and CS80) or thermally-dried sewage sludge (TS20 and TS80) in the cumulative and residual treatments.

	Biomass-C		Biomass-C/TOC	
	Cumulative	**Residual**	**Cumulative**	**Residual**
S0	75.9a	75.9a	1.05bc	1.05ab
MF	82.5a	82.5a	1.42bc	1.42ab
CS20	265.2b	156.5b	1.56c	2.52b
CS80	136.1a	94.9ab	0.44a	1.30ab
TS20	136.1a	86.3a	0.89ab	1.09ab
TS80	89.0a	107.3ab	0.43a	0.88a

Values within the same column followed by the same letter are not significantly different (P≤0.05).

With respect to the unamended and mineral-fertilized soils, SS-amended soils for 3 years feature higher catalase activities (**Figure VIII.1**). Similarly, dehydrogenase and urease activities of soils amended with TS and especially CS are significantly larger than those of unamended and mineral-fertilized soils. These effects are more evident with increasing the amendment rate. The enzyme activities examined of the soils amended once with TS and CS are markedly lower than those of the corresponding soils amended for three consecutive years (**Figure VIII.1**). With the exception of dehydrogenase and urease activities of the soil amended with 80 t ha^{-1} y^{-1} of TS for 1 year, no significant difference in enzyme activities is

Table VIII.5: Correlation coefficients for the cumulative experiments.

	pH	EC	N	TOC	Cbio	Grain yield	Straw yield	Total yield	HI	Spike	Kernel	AW	Cat	DH	Ure
pH															
EC	0.510*														
N	0.648***	0.780***													
TOC	0.706***	0.684***	0.943***												
Cbio	0.524**	0.044	0.251	0.369											
Grain yield	-0.205	-0.533**	-0.315	-0.300	0.196										
Straw yield	0.669***	0.294	0.421*	0.523**	0.459*	0.087									
Total yield	0.440*	0.004	0.207	0.305	0.481*	0.553**	0.842***								
HI	-0.636***	-0.629***	-0.587**	-0.636***	-0.215	0.620**	-0.670***	-0.283							
Spikes	0.254	0.131	0.161	0.245	0.310	0.551**	0.492*	0.695***	-0.021						
Kernel	-0.131	-0.459*	-0.235	-0.234	0.227	0.990***	0.126	0.576**	0.572**	0.587**					
AW	-0.559**	-0.747***	-0.667***	-0.581**	-0.128	0.319	-0.301	-0.078	0.518**	-0.217	0.186				
Cat	0.533**	0.341	0.664***	0.666***	0.276	-0.166	0.612**	0.411*	-0.607**	0.006	-0.106	-0.407			
DH	0.847***	0.631***	0.769***	0.792***	0.419*	-0.361	0.555**	0.303	-0.704***	0.152	-0.299	-0.585**	0.615**		
Urea	0.847***	0.699***	0.838***	0.887***	0.472*	-0.350	0.623**	0.360	-0.727***	0.232	-0.284	-0.595**	0.643***	0.907***	

*,**,*** Indicate significance at the 5, 1, and 0.1% level, respectively. EC = Electrical conductivity; TOC = Total organic C; Cbio = biomass C; HI = Harvest Index; AW = Average Weight; Cat = Catalase; DH = Dehydrogenase; Ure = Urease.

Table VIII.6: Correlation coefficients for the residual experiments.

	pH	EC	N	TOC	Cbio	Grain yield	Straw yield	Total yield	HI	Spike	Kernel	AW	Cat	DH	Ure
pH															
EC	0.321														
N	0.471*	0.384													
TOC	0.277	0.272	0.858***												
Cbio	0.174	-0.200	0.004	-0.001											
Grain yield	0.323	-0.021	0.152	0.039	0.153										
Straw yield	-0.035	-0.146	0.382	0.543**	-0.010	0.045									
Total yield	0.128	-0.080	0.400	0.469*	0.077	0.647***	0.743***								
HI	0.309	0.135	-0.161	-0.335	0.122	0.672***	-0.634***	-0.097							
Spikes	0.369	-0.012	0.038	-0.064	0.198	0.922***	0.007	0.576**	0.641***						
Kernel	0.357	0.036	0.189	0.049	0.172	0.988***	0.041	0.633***	0.673***	0.913***					
AW	-0.157	-0.291	-0.145	0.010	-0.100	0.272	0.057	0.242	0.114	0.223	0.122				
Cat	0.211	-0.007	0.389	0.394	0.045	0.091	0.106	0.104	-0.051	0.028	0.139	-0.255			
DH	0.304	0.003	0.372	0.557**	0.195	0.130	0.476*	0.458*	-0.247	0.096	0.144	-0.072	0.384		
Urea	0.274	0.073	0.383	0.601**	0.106	0.174	0.517**	0.510*	-0.243	0.132	0.177	0.024	0.407*	0.929***	

*,**,*** Indicate significance at the 5, 1, and 0.1% level, respectively. EC = Electrical conductivity; TOC = Total organic C; Cbio = biomass C; HI = Harvest Index; AW = Average Weight; Cat = Catalase; DH = Dehydrogenase; Ure = Urease.

measured between the SS-amended soils as compared to the unamended and mineral-fertilized soils.

Oxidoreductases are involved in oxidative processes in soils. Particularly, catalase has been usually associated with aerobic microbial activity (Rodríguez-Kábana et al 1982). In the cumulative plots, catalase activity is significantly and positively correlated with total N and TOC contents and urease ($P < 0.001$) and dehydrogenase ($P < 0.01$) activities (**Table VIII.5**). Meanwhile, in the residual plots, neither differences between treatments nor significant correlations are detected (**Table VIII.6**). Therefore, the repetitive addition of TS and CS appears to stimulate the synthesis of this enzyme. Some authors (Giusquiani et al., 1995, García-Gil, 2000) has related the increase of catalase activity to an improvement of soil aeration as a consequence of an increase in soil porosity, typical in organically-amended soils with high contents of organic matter (Stevenson, 1994; Tejada et al, 2006).

Generally, enzymes involved in intracellular microbial metabolism, such as dehydrogenase, are good indicators of soil microbial metabolic activities. However, in the present research, dehydrogenase activity is only slightly correlated with soil biomass C content ($P < 0.05$) and only in the cumulative experiments (**Table VIII.5**). Some authors have reported that dehydrogenase activity is inhibited by the toxic effect of heavy metals added with the organic amendments, particularly Pb (Marzadori et al., 1996) and Cu (Chander and Brookes, 1991), but in this case amended soils with high rates (80 t ha^{-1}) have greater dehydrogenase activity than those amended with low rates (20 t ha^{-1}). Consequently, the heavy metal concentrations reached in the soil do not seem to affect the activity of this enzyme and the toxic effect over microbial biomass, previously mentioned, must be attributed to another factor. The decrease of the catalase and dehydrogenase activities observed in the residual experiments could be due to microbial death because substrates are no longer available to sustain microbial biomass or to the fact that intracellular enzyme complexes are degraded by the pool of microorganisms from amended soils (Pascual et al., 1998b; Tejada et al., 2006).

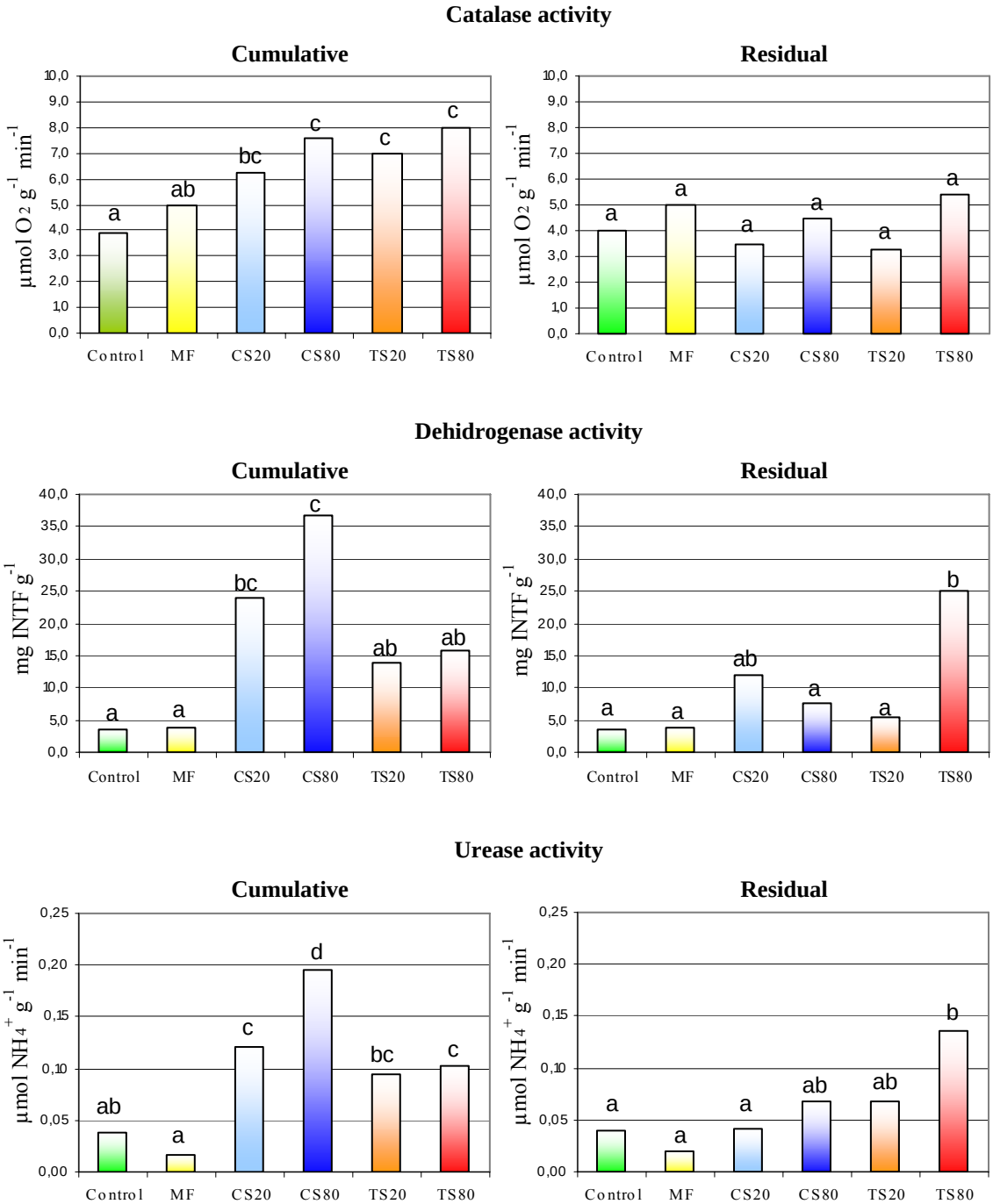

Figure VIII.1: Catalase, dehydrogenase and urease activities of control soil (S0), mineral-fertilized soil, and soils amended with 20 and 80 t ha^{-1} of composted sewage sludge (CS20 and CS80) or thermally-dried sewage sludge (TS20 and TS80) in the cumulative and residual treatments. Bars with the same letter are not significantly different according to LSD test (P=0.05).

Urease is basically an exocellular enzyme of microbial origin (Cecanti and García, 1994). This enzyme catalyses the hydrolysis of urea or ureic type substrates to give CO_2 and NH_4^+ as reaction product. According to García et al., (2000), the presence of substrates and the demand for nutrients by microorganisms or vegetation leads to high activity on this enzyme, involved in the N cycle. That could explain the high levels of urease activity in amended soils in the cumulative experiments as well as the significantly positive correlation with the total N content ($P < 0.001$) and with straw yield ($P < 0.01$) (**Table VIII.5**). Further, in the residual experiment, the greatest urease activity levels match up with the sample with higher contents of N and total dry matter yield, i.e., TS80-R.

VIII.4. CONCLUSIONS

As a whole, the cumulative applications of high doses of CS or TS causes a decrease in soil microbial biomass C content and crop yield, in spite of the high organic matter added, while low doses of SS, especially of CS, show better responses. These results point to a possible deleterious effect on soil fertility due to the use of excessive doses. In contrast, the best results in terms of barley yield are obtained for the soils amended only once with TS and especially CS, which highlight the importance of applying a mature and stable organic matter to the soil.

IX. DISCUSIÓN GENERAL

IX. DISCUSIÓN GENERAL

IX. 1. CINÉTICA DE MINERALIZACIÓN DEL CARBONO

La realización de un experimento de incubación en laboratorio bajo condiciones controladas de humedad y temperatura suministró importante información sobre la dinámica de mineralización del C tanto en el suelo original como en los en los enmendados con lodo compostado o con lodo térmico. Además, los resultados obtenidos fueron ajustados a distintos modelos cinéticos, lo que permitió calcular y comparar parámetros de gran interés, como la fracción de C potencialmente mineralizable de cada una de los suelos y las tasas de mineralización; de esta manera se pueden llegar a conocer con mayor profundidad los posibles beneficios e inconvenientes derivados de la aplicación de estos residuos orgánicos al suelo.

Los resultados obtenidos muestran que los máximos de emisión de C-CO_2 se registran en las primeras 24 horas, debido a las condiciones favorables de humedad y temperatura que provocan la reactivación de la actividad de la biomasa microbiana. Los altos valores registrados para los suelos enmendados con el lodo secado térmicamente, en comparación con el resto de los tratamientos, indican la presencia de una importante cantidad de compuestos fácilmente mineralizables en este residuo y que están presentes en mucha menor proporción en el compostado, probablemente debido a que la mayoría de ellos fueron degradados durante el proceso de compostaje al que fueron sometidos.

Los datos obtenidos se ajustaron a distintos modelos usados para describir la cinética de mineralización del C. Si bien el comportamiento observado para el suelo control y el enmendado con lodo compostado pueden ser descritos adecuadamente por varios de los modelos, el ajuste de los resultados obtenidos para los tres casos es más preciso a medida que aumenta la complejidad del modelo, de manera que el propuesto por Molina et al., (1980), de dos reacciones simultáneas, es el que mejor resultados ofrece; este modelo considera la existencia de dos clases de C orgánico con distinto grado de biodegradabilidad:

una primera fracción formada por compuestos fácilmente mineralizables y una segunda constituida por sustancias más resistentes al ataque microbiano y que se descomponen más lentamente.

Los valores derivados de la aplicación del modelo de dos reacciones simultáneas a los resultados recogidos en este experimento reflejan la mayor presencia de compuestos orgánicos fácilmente biodegradables en los suelos enmendados con lodos secados térmicamente en comparación con el suelo control o con los enmendados con los compostados, donde la fracción fácilmente biodegradable sólo supone entre un 6% y un 11%, respectivamente, del C mineralizable total. Además, los valores de la tasa de mineralización de esta etapa calculados para el suelo con lodo térmico son anormalmente altos y similares a otros obtenidos previamente para suelos enmendados con lodos que no han sido sometidos a ningún tratamiento; esto corrobora la falta de estabilización de la materia orgánica presente en los lodos secados térmicamente. Al mismo tiempo, otros parámetros calculados para ambas fases sugieren que la materia orgánica del suelo control y la del enmendado con lodo compostado se mineraliza de manera similar durante la primera fase, mientras que durante la segunda, la materia orgánica adicionada con éste lo hace algo más rápido al estar menos humificada que la existente en el suelo. Los valores de tasa de mineralización inicial (C_2K_2) y los tiempos de vida media ($t_{1/2}$) obtenidos confirman estos resultados.

El cálculo del coeficiente de mineralización complementario permite estimar que alrededor de un 25% del C orgánico aportado al suelo con el lodo secado térmicamente es mineralizado tras los primeros 60 días, frente al 3% del aportado por el compostado. Así mismo, los coeficientes diarios de mineralización complementarios obtenidos muestran que la materia orgánica de los suelos enmendados con lodos térmicos sigue activa, experimentando una mineralización intensa incluso pasados los primeros 60 días de incubación.

Puede decirse que los suelos enmendados con lodos secados térmicamente muestran una mayor tasa de mineralización del C debido a la inestabilidad de su materia orgánica y a los procesos de degradación, que como consecuencia tienen lugar en el suelo con las

condiciones de temperatura y humedad adecuadas. En cambio, las emisiones de C-CO_2 de los suelos enmendados con lodos compostados son mucho menores debido a la estabilización y maduración que experimenta la materia orgánica durante el proceso previo de compostaje. Además, debido a su tasa de mineralización menor, la materia orgánica del lodo compostado puede dar lugar a un incremento mayor de la cantidad de humus estable en el suelo, lo que ejercerá una influencia muy positiva en la calidad del mismo, mientras que la materia orgánica más activa aportada con los lodos secados térmicamente es de esperar que genere un mayor suministro de nutrientes para la planta.

Si el objetivo es restaurar la materia orgánica del suelo rápidamente sería más recomendable el uso de lodos compostados, con una materia orgánica más estable y madura; por el contrario, si es promover a corto plazo la reactivación de los microorganismos del suelo y el aporte de nutrientes, el uso de lodos secados térmicamente como fertilizantes puede ser más apropiado, aunque ha de tenerse en cuenta que la aplicación de enmiendas inestables e inmaduras puede resultar negativa para el crecimiento de las plantas y las propiedades del suelo ya que, entre otros efectos adversos, puede incrementar la tasa de mineralización del C orgánico del suelo a través de una intensa oxidación microbiana e inducir la aparición de condiciones anaerobias.

IX.2. EFECTOS SOBRE LAS PROPIEDADES QUÍMICAS DEL SUELO

Dentro del experimento de campo llevado a cabo durante tres años consecutivos, se estudiaron los efectos acumulativos y residuales de los lodos compostados y los secados térmicamente sobre las propiedades fisicoquímicas y químicas del suelo, centrándose la atención en las transformaciones de la materia orgánica y sus fracciones humificadas.

La aplicación de los lodos al suelo induce una serie de modificaciones en las propiedades del mismo derivadas de sus características intrínsecas, que se acentúan con las sucesivas aplicaciones: aumento del pH (aunque en límites aceptables), de la salinidad (alcanzando niveles que pueden llegar a tener efectos fitotóxicos y deben ser controlados) e incrementos notables de los contenidos de N y P, resultados característicos de suelos

enmendados con lodos de depuradora, ya que estos residuos son normalmente muy ricos en estos elementos.

Con respecto al suelo control, las aplicaciones de ambos tipos de lodos incrementan de manera significativa los contenidos de C en todas las fracciones analizadas: C orgánico total, C orgánico extraíble, C de los ácidos húmicos y C de los ácidos fúlvicos. Como es esperable, estos incrementos son mayores a medida que aumentan las dosis (de 20 t ha^{-1} a 80 t ha^{-1}) o el número de aplicaciones realizadas. Sin embargo, a pesar del mayor contenido de C orgánico total en el lodo secado térmicamente (casi el doble que el del compostado), en los suelos enmendados con este último, los contenidos de C son mayores en todas las fracciones analizadas, incluida la de C orgánico total. En base a los resultados obtenidos en el experimento de incubación descrito anteriormente, este hecho puede deberse a que la materia orgánica de los lodos secados térmicamente es rica en compuestos fácilmente biodegradables y experimenta una intensa mineralización en el suelo. Esto se traduce en unos contenidos finales de C orgánico total y de sus fracciones inferiores a los esperables y menores que los de los suelos enmendados con el lodo compostado, cuya materia orgánica es más estable y su cinética de mineralización más lenta.

En el caso de los tratamientos residuales, los contenidos de C de las distintas fracciones analizadas tienden a decrecer a medida que pasa el tiempo desde la aplicación de la única enmienda, alcanzándose niveles similares entre los suelos enmendados con uno u otro tipo de lodo. El descenso de C es especialmente marcado en el caso de los ácidos fúlvicos, probablemente debido a la elevada degradabilidad de muchos de los compuestos presentes en esta fracción y/o a su transformación en moléculas más complejas como, por ejemplo, ácidos húmicos. Por otra parte, el incremento observado de los índices de humificación (como el porcentaje de ácidos húmicos o el grado de polimerización), indican la existencia de una materia orgánica humificada y compleja, lo que supone un resultado positivo en el contexto del reciclaje de estos residuos en agricultura.

IX.3. EFECTOS SOBRE LAS PROPIEDADES ESTRUCTURALES Y FUNCIONALES DE LAS SUSTANCIAS HÚMICAS DEL SUELO

Profundizando en el estudio de la materia orgánica, se extrajeron los ácidos húmicos de ambos lodos, así como los de los suelos enmendados con ellos (en dosis acumulativas de 80 t ha^{-1} durante tres años) y los del suelo sin enmendar. Se analizaron mediante diversas técnicas químicas y espectroscópicas lo que permitió determinar comparativamente las propiedades moleculares, estructurales y funcionales de los ácidos húmicos de ambos lodos y evaluar los efectos de su aplicación sobre los del suelo.

Al comparar los resultados del análisis de la composición elemental, los ácidos húmicos del lodo compostado muestran mayor relación O/C y menor H/C y N/C que los del secado térmicamente, que presenta unas estructuras con un elevado contenido de H, algo propio de cadenas largas con alto grado de saturación; por otra parte, el alto porcentaje de N encontrado puede deberse a la incorporación de residuos de naturaleza proteica. Los ácidos húmicos del suelo control presentan mayores contenidos de O y C y menores de H, N y S que los tratados con lodos; sin embargo, la incorporación sucesiva de los residuos al suelo supone un incremento de los niveles de todos estos elementos, con la excepción del O, que decrece, lo que indica un menor grado de oxidación de la materia orgánica presente en los suelos enmendados.

La espectroscopía infrarroja pone de manifiesto el marcado carácter alifático de los ácidos húmicos del lodo secado térmicamente y su alto contenido en compuestos de tipo polisacárido y grupos nitrogenados, probablemente debido a la presencia de restos de cadenas polipeptídicas; estas características aparecen en mucho menor grado en los del lodo compostado y apenas se encuentran en los del suelo control. Sin embargo, los ácidos húmicos de los suelos enmendados muestran intensidades crecientes en zonas asociadas con la presencia de estructuras alifáticas, grupos amida y componentes polisacáridos. Estos efectos son más evidentes en el caso del tratamiento con lodo secado térmicamente e indican que la aplicación de ambos residuos afecta, al menos parcialmente, a la estructura y a las propiedades funcionales de los ácidos húmicos del suelo original.

La fluorescencia de emisión, excitación, barrido sincrónico y de tres dimensiones (matrices de excitación-emisión) muestra para los ácidos húmicos de ambos lodos la presencia de picos de baja intensidad en zonas de longitud de onda corta, atribuibles a un bajo nivel de cromóforos conjugados, lo que sugiere que los componentes estructurales de estas sustancias húmicas son poco complejos, de pequeño tamaño molecular y bajo grado de aromaticidad y, por tanto, poco humificados. Por el contrario, los ácidos húmicos del suelo control muestran picos intensos en zonas de longitud de onda alta, lo que se asocia a la presencia de moléculas altamente conjugadas y con un marcado grado de humificación. Sin embargo, los espectros de fluorescencia de los ácidos húmicos correspondientes a los suelos enmendados con ambos lodos muestran propiedades intermedias entre los del suelo control y los de los lodos empleados, lo que apunta hacia una incorporación parcial y progresiva a los ácidos húmicos del suelo de componentes poco humificados provenientes de los residuos. Este efecto es más evidente en el caso de los lodos secados térmicamente, cuyos ácidos húmicos, como se ha comentado anteriormente, son especialmente ricos en estructuras más simples.

El uso de técnicas termogravimétricas, novedoso en el seguimiento de la evolución de los ácidos húmicos del suelo, muestra marcadas diferencias entre el comportamiento térmico de los del lodo secado térmicamente y los del suelo control o los del lodo compostado, más similares entre ellos. Estas grandes diferencias se deben principalmente a la alta proporción de carbohidratos y estructuras alifáticas presentes en la composición de los ácidos húmicos del lodo secado térmicamente, que además apenas tienen estructuras aromáticas, mucho más presentes en los del compostado y en especial en los del suelo control. Al cabo de tres años de sucesivas aplicaciones, los ácidos húmicos de los suelos enmendados con ambos lodos presentan un comportamiento térmico bastante parecido a los originales del suelo, pero llama la atención la persistencia de restos de estructuras proteicas o de otro tipo de compuestos con N, especialmente en el tratamiento con el lodo secado térmicamente.

La espectroscopía de resonancia magnética nuclear (RMN) proporciona gran cantidad de información de elevada precisión en el estudio y caracterización de los ácidos húmicos de los lodos de depuradora y sus efectos sobre los del suelo. Los resultados obtenidos confirman muchos de los puntos observados con anterioridad con las otras técnicas. Así, se identifica la

importante presencia de cadenas de metil-C, metilen-C y otros compuestos alifáticos con grupos carboxílicos en los ácidos húmicos del lodo secado térmicamente, así como su pobreza en compuestos aromáticos, al contrario que los del compostado y en especial que los del suelo control. La contribución más significativa de ambos lodos a la composición de los ácidos húmicos del suelo original es la incorporación de compuestos de carácter alifático que se incrementa en los suelos enmendados con el secado térmicamente y con el compostado en un 13.3% y un 15.8% respectivamente. Además, aunque de menor importancia, también debe considerarse la incorporación de estructuras orgánicas nitrogenadas simples, lo que podría explicar en parte el aumento de las relaciones H/C y N/C y el descenso en los contenidos de O discutidos anteriormente.

Para profundizar en el estudio de las propiedades ácido-base de las sustancias húmicas (ácidos húmicos y fúlvicos), se realizaron una serie de titulaciones potenciométricas cuyos resultados se interpretaron con la ayuda del modelo NICA-Donnan. En comparación con los ácidos húmicos y fúlvicos del suelo, los del lodo compostado y en especial los del secado térmicamente tienen menores contenidos en grupos funcionales ácidos, menor heterogeneidad de grupos de tipo carboxílico y fenólico y mayor afinidad protónica. La incorporación al suelo de los lodos provoca un descenso de los contenidos en grupos funcionales ácidos y un ligero incremento de las afinidades protónicas de los ácidos húmicos y fúlvicos. Estos efectos son más evidentes en las sustancias húmicas de los suelos enmendados con lodos compostados que con los secados térmicamente; se puede decir que el proceso de compostaje, en comparación con el de secado térmico, produce fracciones de ácidos húmicos y fúlvicos con propiedades ácido-base más similares a las que se pueden encontrar de manera natural en el suelo control y por tanto, inducen menores modificaciones en sus compuestos húmicos.

Después de tres años de aplicación de ambos lodos, la contribución de éstos a la estructura de los ácidos húmicos del suelo se evidencia a través de los cambios en la funcionalidad, estructura molecular y composición de los mismos; en general, los de los suelos enmendados, especialmente con los lodos secados térmicamente, muestran mayor cantidad de componentes alifáticos y residuos proteínicos. Es de destacar que aunque existen diferencias entre los ácidos húmicos de los suelos enmendados con uno u otro lodo, no son

tan marcadas como las existentes entre los ácidos húmicos originales de ambos. Esto se encuentra en clara relación con lo visto en el experimento de mineralización y que se ha comprobado al analizar las distintas fracciones de la materia orgánica: el lodo térmico experimenta una intensa mineralización al ser aplicado al suelo, lo que se traduce en una maduración y estabilización, al menos parcial, de la materia orgánica que aporta, lo que se refleja en que las estructuras de los ácidos húmicos de los suelos enmendados con lodos térmicos no son tan distintas, cómo cabría esperar, respecto a las de los tratamientos con los compostados.

IX.4. EFECTOS SOBRE LAS PROPIEDADES BIOLÓGICAS Y BIOQUIMICAS DEL SUELO

La biomasa microbiana y las actividades enzimáticas del suelo juegan un papel clave en los ciclos biogeoquímicos de los nutrientes, catalizando numerosas reacciones bioquímicas que ejercen una gran influencia sobre la disponibilidad de elementos esenciales para el cultivo, y en consecuencia sobre la productividad del sistema. Por ello, en el experimento de campo también se prestó atención a los efectos residuales y acumulativos que la aplicación de los lodos ha producido sobre algunas de las propiedades biológicas y bioquímicas más importantes del suelo.

La aplicación de los lodos al suelo supone una reactivación general de la actividad microbiana debido a la incorporación de sustratos orgánicos fácilmente biodegradables que son utilizados como fuente de energía; sin embargo, a pesar de que los contenidos más altos de C de la biomasa microbiana corresponden a los suelos enmendados repetidamente con dosis bajas de lodo (20 t ha^{-1}), si es referido al C orgánico total del suelo, se observa que los mejores resultados se obtienen en los que sólo recibieron una dosis inicial de residuo, lo que puede estar relacionado con posibles efectos negativos derivados de un exceso del mismo en el suelo y que pueden estar vinculados a diversos factores, como variaciones en las condiciones redox y la salinidad o la presencia de metales pesados.

Sin embargo, a pesar de los bajos contenidos de C de la biomasa microbiana detectados, las actividades enzimáticas analizadas (catalasa, deshidrogenasa y ureasa) son marcadamente más altas en los suelos correspondientes a los tratamientos acumulativos que en los que recibieron una única enmienda inicial. Es especialmente llamativo el caso de la deshidrogenasa, una enzima intracelular considerada como un buen indicador de la actividad oxidativa de la biomasa microbiana, sensible a la presencia de metales pesados y que muestra mayor actividad en los suelos enmendados con las dosis altas que con las bajas. Por otra parte, tanto la enzima catalasa (asociada a los procesos aeróbicos de los microorganismos) como la ureasa (involucrada en el ciclo biogeoquímico del N) también presentan una actividad creciente con la aplicación de mayores cantidades de residuo al suelo; esto puede ser debido a una mejora de las condiciones físicas del suelo en el primer caso y a un incremento de los contenidos de N en el segundo.

IX.5. EFECTOS SOBRE EL CULTIVO

Con el fin de realizar una valoración agronómica del uso de estos lodos de depuradora como enmiendas, se analizaron distintos parámetros relacionados con el rendimiento y la calidad de la cebada cultivada anualmente en las parcelas experimentales durante los tres años. En general, los mejores índices de rendimiento de cultivo corresponden a los tratamientos residuales, especialmente con lodo compostado, que muestran una clara mejora respecto a los suelos sin enmendar y al que recibió fertilización mineral,

En los tratamientos acumulativos, aumenta la cantidad de paja obtenida, pero otros parámetros como el índice de cosecha o el peso del grano son más bajos incluso que los obtenidos para el suelo sin enmendar, lo cual puede estar asociado a un exceso de N añadido con ambos tipos de lodo. Estos resultados subrayan la importancia de aplicar al suelo una materia orgánica estable y madura (cuya eficacia queda demostrada) y de controlar otros factores como el pH, la salinidad, el N o los niveles de metales pesados en el suelo.

X. CONCLUSIONES

X. CONCLUSIONES

Los resultados de este estudio permiten obtener las siguientes conclusiones generales:

1. El lodo secado térmicamente presenta una materia orgánica rica en compuestos fácilmente biodegradables, que son descompuestos de forma intensa en el suelo mientras que en los suelos enmendados con lodos compostados la mineralización de la materia orgánica es mucho menor ya que el compostaje favorece su estabilidad.

2. En general, la adición de lodos de depuradora produce un incremento de los contenidos de materia orgánica del suelo y de las sustancias húmicas, así como una mejora de los parámetros de humificación.

3. Existen diferencias sustanciales entre la composición química y la funcionalidad de los ácidos húmicos de ambos lodos; los compostados presentan estructuras más policondensadas, oxidadas, insaturadas y aromáticas que las de los secados térmicamente; a la vez, ambos muestran menor grado de aromaticidad, policondensación y polimerización que los originales del suelo.

4. Después de tres años de aplicación de ambos tipos de lodos, su contribución a la estructura de los ácidos húmicos del suelo se evidencia a través de los cambios en la funcionalidad, estructura y composición molecular de los mismos, con una mayor presencia de componentes alifáticos y residuos proteícos, especialmente en los enmendados con el lodo secado térmicamente.

5. A pesar de la desigualdad entre los ácidos húmicos de los suelos enmendados con uno u otro tipo de lodo, las diferencias no son tan marcadas como las existentes entre los ácidos húmicos originales de ambos residuos debido a una estabilización *in situ* de la materia orgánica adicionada con el lodo térmico.

6. La aplicación de dosis excesivas de ambos lodos provoca un descenso de la biomasa microbiana del suelo pero favorece la reactivación de enzimas implicadas en ciclos biogeoquímicos de nutrientes en el sistema, sobre todo en los suelos enmendados con lodos compostados.

7. La aplicación de dosis adecuadas de lodos, especialmente compostados, tiene efectos beneficiosos sobre los rendimientos de cultivo de cebada, si bien es preciso tener en cuenta los factores limitantes en su utilización y manejo para prevenir efectos adversos.

En definitiva, ante la problemática que genera la creciente producción de lodos de depuradora y los bajos contenidos de materia orgánica existentes en una gran mayoría de los suelos pertenecientes a ecosistemas agrarios áridos y semiáridos mediterráneos, el uso de los lodos compostados o secados térmicamente como enmiendas orgánicas supone una alternativa interesante desde el punto de vista ambiental y agronómico. Mientras que la aplicación de los lodos compostados resulta adecuada para restaurar eficazmente la materia orgánica del suelo en un breve espacio de tiempo, el empleo de lodos secados térmicamente puede ser más apropiado para promover a corto plazo la reactivación de los microorganismos del suelo y el aporte de nutrientes, teniendo siempre en cuenta los factores limitantes existentes.

XI. BIBLIOGRAFÍA

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